Optimising onion (*Allium cepa* L.) bulb quality for counter-seasonal export markets

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Declaration of originality

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Statement of co-authorship

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Abstract

Onion bulbs cultivated in the Southern Hemisphere for counter-season, Northern Hemisphere markets must be of high quality at the point of presentation to the consumer. Consistently achieving this requires an in-depth understanding of the interaction between pre- and post-harvest factors that affect bulb quality and storage life. This industry-focussed study specifically investigated key factors that affect storage life (sprouting) in Tasmanian, Australia, grown bulbs. These factors were identified in consultation with industry and a review of the scientific literature.

Onion bulb production in Tasmania, a cool-temperate region, is a low cost production system that takes advantage of the region's long dry summers that enable bulbs to be lifted out of the ground (termed "lifting") prior to complete canopy senescence and field curing in windrows. The timing of lifting based on plant growth and development events associated with lodged crop canopies (termed "tops-down") was one of the pre-harvest factors investigated in this study; 80% tops-down is used commercially as an indicator of optimal bulb development for lifting. Internal sprout growth and respiration rate during long term storage were generally lowest in bulbs lifted at *ca* 90% tops-down and increased with early or late lifting treatments. These data support the recommendation that to optimise bulbs for long term storage, lifting should be changed from 80% to *ca* 90% tops-down

While 80% tops-down is used by industry as a marker to determining time of lifting, forecasting this event to schedule lifting operations is difficult, because it is not known how to predict the time at which foliage lodging begins, or the length of time to 80% tops-down.

Changes in the mechanical properties of pseudostem bases were evaluated using a custom-built tool, which measured stem ovalisation under transverse load as a proxy for susceptibility to structural failure via Brazier buckling. Softening of tissues at the pseudostem base was found to precede and govern canopy collapse within and among cultivars. Monitoring of this softening provided a pre-lodging indicator of the development progression to pseudostem failure (tops-down) independent of genotype and environment.

Post-harvest factors investigated include the effect of mechanical impacts during handling on bulb respiration rate, weight loss and sprout growth. Cured bulbs from different crops sampled during commercial grading were found to have different respiration rates prior to handling, demonstrating the interaction between pre- and post-harvest factors. In subsequent controlled impact experiments, higher respiration rates and faster sprout growth were recorded when bulbs were impacted on the base plate, and also with greater impact magnitudes. Minimising the force of impacts, and avoiding impacts to the base plate are thus recommended as best practice for the handling of onions intended for long term storage and the sensitivity of bulbs to impacts in future studies should control impact location.

Lifting the crop at *ca* 90% tops-down reduces sprout growth and respiration rates during long term storage. To enable prediction of this important stage, this study demonstrates that foliage collapse is both preceded and governed by softening of the neck tissues, which leads to structural failure via Brazier buckling. Measuring deformation at a constant load may be used to estimate a crops susceptibility to lodging, and may lead to the development of predictive models. Finally, storage life can also be increased by minimising mechanical

impacts, particularly to the base plate, which increases respiration rate and weight loss, and accelerates sprout growth.

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"If I have seen further it is by standing on the shoulders of giants" (Isaac Newton, 1676).

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List of abbreviations

ABA	Abscisic acid
ETc	Evapotranspiration
ESWM	Estimated self-weighted moment
N	Newton
DAP	Days after planting
DW	Dry weight
Pre-topping	Packing line bulb sampling point prior to removal of stem and root material
Post-topping	Packing line bulb sampling point after the removal of stem and root material
Post-grading	Packing line bulb sampling after size grading
Tops-down	Developmental stage at which the canopy of an onion crop collapses
VPD	Vapour pressure deficit

Preamble

This project was initiated by industry partners, Field Fresh Tasmania (Australia's dominant onion exporter), to address challenges associated with the reliable supply of high quality bulbs that meet Northern Hemisphere market requirements. The scope of the research reported herein was based on the direction provided by the industry partners. This thesis comprises an introduction and thesis scope (Chapter 1), four research chapters (2-5) and a final discussion chapter (Chapter 6). The four research chapters address knowledge gaps on factors that affect bulb storage life including physical impacts during handling (Chapter 2); crop maturity at the time of lifting (Chapter 3); mechanism of canopy collapse (Chapters 4) and variation in canopy collapse among cultivars (Chapter 5). In chapter 6, the results from all chapters are discussed and synthesized and major findings and conclusions are highlighted. Further, recommendations on how to minimize the impacts of pre- and post-harvest physiological factors on onion bulb storage are presented. The references cited within chapters have been placed at the end of the thesis.

Chapter 1: Introduction and thesis scope

Abstract

Onion production in Tasmania to supply counter-seasonal markets in Europe makes an important contribution towards the local agricultural economy. To supply these markets, the industry is required to produce high quality (termed 'Class One') bulbs at low cost. Although the industry is currently based on onion cultivars characterised by a long storage life the reliable production of high-quality, durable bulbs with a long storage life remains a key challenge for the industry. To address this challenge, a better understanding is needed of how pre-and post-harvest factors and their interactions influence onion bulb quality. This chapter outlines current commercial onion production practices and knowledge gaps relating to bulb quality, and identifies pre and post-harvest research opportunities within the context of the Tasmanian production system.

Background

Onions are one of the world's most popular vegetables and have been cultivated for their intrinsic properties for more than 4000 years (Hanelt 1990). They are thought to have been originally domesticated in the mountains of Turkmenistan and Northern Iran (Brewster 2008) whilst the earliest records available are depictions from Egypt dating back to 2700BC (Hanelt 1990). Now, onions are grown in regions ranging from the subtropics to the subarctic, but are best adapted to subtropical and temperate regions (Brewster 2008). Onions are a versatile vegetable used worldwide to add flavour to an extensive variety of cuisines. Such is the importance of onion bulbs in India that Time magazine reported a price hike for onions in 2013 threatened to destabilise the government (Bhowmick 2013). Onions are also valued for their purported health benefits, some of which have been attributed to the presence of organosulphur compounds (Keusgen 2002). Together, the onions culinary versatility, flavour, long storage life and adaptation across agro-ecosystems has made it one of the world's most popular vegetables, and the consequent demand makes it a regularly traded commodity in both domestic and global markets.

Several other members of the genus *Allium* are also widely valued as food crops including garlic (*Allium sativum.* L), leek (*Allium ampeloprasum.* L), chives (*Allium schoenoprasum.* L), and Japanese bunching onions (*Allium fistulosum.* L). This project will focus solely on bulbing onions (*Allium cepa*. L), cultivated for the fleshy bulb with dry outer layers (skins) and no visible green tissue. These are sometimes referred to as dry

onions by the United Nation's Food and Agriculture Organisation (FAO) when quantifying world production.

Worldwide production

The last half century has seen an exponential growth in the world production of onion bulbs at a rate of approximately 3.6% per year, from 15 million tonnes in 1960 to 82.9 million tonnes from 4.2 million hectares in 2012 (FAO 2014).

Table 1-1. Onion bulb production across continents in 2013 (FAO 2014)

Continent	Production area (1000's ha)	Total production (1000's tonne)
Australia	5.5	301.7
Africa	659.2	9,650.1
Asia	3,121.4	57,312.8
Europe	362.9	9,224.9
North America	63.1	3,351.5
South America	160.9	4,283.2

Australia produces approximately 0.35% of the world's onion bulbs (FAO 2014). In 2012, the country produced 346,000 tonnes from 6,708 hectares (Australian Bureau of Statistics 2013). Approximately one third of this volume was produced on the southern island State of Tasmania. Another third was produced in the State of South Australia with the remaining third of production split mainly between the States of Victoria, Queensland and Western Australia (Australian Bureau of Statistics 2013). Onion production in Tasmania is primarily geared towards supplying the counter-seasonal demand from Northern

Hemisphere markets such as the United Kingdom and Germany. They compete in these markets with local supply and alternative counter season supplies from New Zealand, Africa and South America. In contrast, the other Australian States focus on year-round supply to the domestic markets (DPIPWE 2014).

Onion growth and development

A detailed qualitative description of onion development during the first year of its biennial lifecycle was published in French by Rey et al. (1974), and later in English by Brewster (2008). Crops can be established from seeds, seedlings or bulblets/sets (Brewster 2008). Direct seeding has the lowest establishment costs and the longest growth time while seedlings and bulblets increase the cost of establishment but require less time to reach maturity in the field (Brewster 2008). Established, vegetative plants begin forming bulbs in response to photoperiod and temperature (Lancaster et al. 1996; Steer 1980). The bulb is formed by multiple layers of swollen leaf sheaths that partially enclose the compressed stem. The outermost leaf sheath layers desiccate to form dry sheath tissue (skin), a process termed curing. Curing occurs either in the field in regions of low rainfall (before or after lifting) or in bulk storage with forced air (sometimes heated) in regions where rainfall may cause staining of skins and increased post-harvest disease. Provided that sufficient quality has been achieved, bulbs are then stored and distributed to consumers during their dormant phase, the length of this phase varying among cultivars. The reproductive stage of development follows the breaking of dormancy (sprouting), and is relevant commercially for seed production. Dormancy can divide in to endo-dormancy

(dependent on the bulb) and eco-dormancy (dependent on the external environment) (Chope et al 2012), An early transition to the reproductive phase is undesirable during bulb crop growth (sometimes referred to as bolting) or bulb storage. Transition to the sprout growth stage during storage results in market rejection due to the presence of externally visible sprouts and changes in bulb shape (Tanaka et al. 1985).

Onion bulb production in Tasmania

Most of Tasmania's onion bulb production is carried out in the cool-temperate regions of the North and North West. Crops are established from seed sown using precision planters. The growing season extends from May to March, depending on the photoperiod requirement of the onion cultivar (DPIPWE 2014). For example, the cultivar '*Early Creamgold'* is planted in May and June while '*Regular Creamgold'* is planted from July to September. Some F1 hybrid cultivars are planted as late as early October. Following a period of growth where leaf blades are formed and bulbs are initiated, crop lifting operations start in late December when approximately 80% of the crop canopy has lodged, followed by 3-4 weeks of infield curing. Bulbs are then harvested, at which time the majority of leaf material is removed, and transported to packing facilities where they are placed in ventilated storage. Prior to grading, the remaining neck material and roots tissues are removed. Bulbs are then graded by size and quality, and packed into containers either loose, or in bags ready for export. The journey from Tasmania to Europe by containerised sea freight takes 4-6 weeks during which bulkhead fans provide ventilation

with ambient air. On arrival in Europe, bulbs may be stored before being regraded and packaged for delivery to retail outlets.

Tasmanian export industry

An estimated 85% of the Tasmanian onion crop, worth approximately $27 million, is exported each year to the European markets (DPIPWE 2014). According to the standards set by the United Nations Economic Commission for Europe (UNECE) for the marketing and quality of onion bulbs (FFV-25), bulbs must arrive in 'Class One' condition, having at least one entire skin, no disease, have no externally visible sprouts and must also meet strict quarantine standards. Post-harvest diseases that can lead to customer rejection include neck rot (*Botrytis allii*) and black mould (*Aspergillus niger*). Additional requirements may also be specified by specific markets such as size ranges and colour. Although it is possible for growers in Europe to supply out of season onion bulbs, this requires the use of controlled atmosphere refrigerated storage and/or sprouting inhibitors such as Maleic Hydrazide (MH). Recent work has also suggested Ethaline as alternative sprout suppressant (Chope et al 2012). The use of these technologies comes at considerable financial cost and there is concern about the potential health impact of MH sprouting inhibitors, with some markets preferring untreated bulbs (Downes et al. 2010). By producing bulbs in the Southern Hemisphere and delivering them to Northern Hemisphere markets at low cost, Tasmanian exporters can compete with European sourced bulbs which have been held in storage.

The ongoing success of the onion export industry in Tasmania in a competitive global market depends on its ability to consistently and economically produce robust, high-quality bulbs with long storage life. To achieve this production objective, there is a need to develop and refine appropriate agronomic, and postharvest handling and storage practices based on an extensive knowledge of bulb physiology, with a focus on key pre- and post-harvest factors that affect bulb quality. Failure to effectively manage these factors may result in low or variable bulb quality, compromising market access.

Pre-harvest factors affecting bulb quality

Key pre-harvest variables have been shown to influence bulb quality and yield; these include day length, nutrition, irrigation and time of lifting.

Day length

Day length is a key determinant of bulb development and onion cultivars are generally categorised by the photoperiod requirement to initiate bulbing (Lancaster et al. 1996). The shorter the day length requirement the closer to the equator onion bulbs can be reliably cultivated. As a guide, cultivars are categorised into short (>11-12 h), intermediate (>13-14 h) and long (>16 h) day lengths (Brewster 2008). In addition to day length there is also a minimum heat unit accumulation (day degree) requirement for the initiation of bulbing which varies among cultivars (Lancaster et al. 1996; Steer 1980). Planting of crops in regions located at the margins of the required day length and temperature requirements may lead to significant reductions in yield and quality, as they may fail to produce bulbs or transition to bulbing too early, producing small unmarketable bulbs (Lancaster et al. 1996).

Water and nutrient management

Onions have a relatively shallow root system in comparison with other crops, and the majority of roots are confined to the top 20 cm of soil (de Melo 2003). This limits the depth from which they can access available soil water and nutrients and influences crop management. In terms of soil water, Shock et al. (2000) compared the performance of a

drip-irrigated treatment plots in a onion crop over a range of soil water potentials and concluded that a soil water potential of -20 kPa at 20 cm soil depth was optimal for maximising yield and for minimising the risk of nutrient leaching. Similarly Enciso et al. (2009) found that soil water potentials as low as -30 kPa or 75% ETc (evapotranspiration) could be used without incurring any yield penalty. Once bulbs reach the curing phase high moisture levels are associated with bulb rots in storage. It is therefore routine practice to monitor soil water availability, schedule irrigation accordingly during the growth phase, and cease irrigation prior to bulbs curing.

In commercial onion production, available mineral nutrition is managed via fertiliser programmes based on regular soil nutrient testing. In particular, nitrogen is the most studied nutrient in onion crop management due to the complexities of the nitrogen cycle, brought about by mineralisation, volatilisation and leaching, leading to a relatively inefficient uptake of nitrogen by the onion root system (Halvorson et al. 2002). In addition to affecting onion growth, nitrogen has been found to reduce the time taken for plants to develop the lodging stage of development (Mondal et al. 1986b) and excessive nitrogen application, particularly during late crop growth can lead to high incidence of rot during storage (Wright 1993). Undersupply results in lower yields (Boyhan et al. 2007). Other nutrients have been studied but to a lesser extent. Commercially, phosphorus, potassium and sulphur along with other mineral (macro and micro) requirements are applied pre-planting and incorporated into the soil. Sulphur nutrition had been shown to effect on flavour and pungency of onions, and is therefore of particular importance (McCallum et al 2005). Further phosphorus is banded below the seed during drilling to

promote root growth. In contrast, nitrogen is applied in two to three top dressings after crop establishment.

Timing of lifting

Onion bulbs grown in cool-temperate climates are lifted out of the soil before leaf senescence is complete and skins have finished curing. This process is the most profound pre-harvest management intervention to affect a developing crop, isolating the plants from both soil water and nutrients. The timing of lifting during the maturation of onion crops has been shown to effect a range of crop parameters including total yield (Sargent et al. 2001), number of intact outer skins (Wright et al. 2001), sprouting (Komochi 1990), dry matter, amino-nitrogen concentration (Nilsson 1980) and incidence of rot during storage (Wright et al. 2001), which together influence the final marketable status of bulbs. The removal of leaf bade material prior to curing (green topping), is an additional intervention has been used in some production systems during this period. However it is also associated with an increased incidence of rots (Wright et al. 2001) and is not in common use within the Tasmanian onion production system.

The decision on when to lift bulbs is generally considered to be a trade-off between yield and quality. A range of recommendations as to the optimal time of lifting have been published, varying with climate, cultivar, crop management, post-harvest storage, or the methods used to assess lifting time treatments (Komochi 1990; Nilsson 1980; Sargent et al. 2001; Wright et al. 2001; Wright 1997). In Tasmania, ‘early’ and ‘late’ lifting is

associated with a reduction in yield and post-harvest quality, respectively (Gracie 2006). Coinciding with tops-down and curing is the transition from bulb growth to bulb dormancy. Though the mechanisms which control bulb dormancy remain unclear the timing of lifting appears to be key to maintaining dormancy and therefore quality in onion crops grown for long term storage (Suojala 2001).

Post-harvest factors affecting bulb quality

Once bulbs have been harvested and skin colour stops developing, there are limited opportunities for improving bulb quality. Therefore, the focus of management is to retain bulb quality by a greater understanding of post-harvest factors that affect the rate at which the quality is lost; these factors include temperature, relative humidity, and the physical handling of bulbs.

Storage environment

As extensively reported for many fruits and vegetables (Bartz et al. 2003; Eskin et al. 2010), temperature, and vapour pressure deficit of the storage environment are key post-harvest factors affecting the shelf life of onions. The effect of temperature on bulb dormancy has been described in detail by Abdalla et al. (1963). By storing bulbs *'Excel'* at a range of temperatures and for a range of times, Abdalla et al. (1963) found that dormancy was shortest at 15 °C and longest at 0 or 30 °C. The long-term cold storage of onion bulbs comes at a significant economic cost from maintaining and running the cold storage infrastructure. In onion storage systems without temperature control it has been

recommended that the relative humidity is maintained at 65-70% to minimise weight loss and maintain skin flexibility (Brewster 2008). Managing temperature and relative humidity in storage to satisfy market quality requirements can be challenging and is energy intensive. To limit transport and storage costs, bulbs are currently shipped from Tasmania to European markets in containerised freight at ambient temperatures with ventilation supplied by bulkhead fans throughout transport ('Fantainers'). Although control of the storage environment is limited, the economy of this approach allows Tasmanian producers to be competitive in European markets.

Mechanical impacts

Labour costs are a comparative disadvantage in Australia, with the high relative cost of labour in Tasmania necessitating that grading and packing is largely carried out by mechanised bulb handling systems which, whilst cost effective, result in a greater level of mechanical impact. Mechanical impacts have been shown to reduce the quality of onion bulbs (Herold et al. 1998; Timm et al. 1991). Herold et al. (1998) assessed the respiration response and storage loss of onion bulbs '*Rijnsburger Balstora'* after subjecting these to a range of mechanical loads by dropping bulbs from heights of 17.5 - 105 cm on to a conveyor belt or steel plate. These mechanical loads increased respiration rate both immediately and during the 11 week assessment period. The authors also established a positive relationship between drop height onto a steel plate and loss of bulb quality due to sprouting. Other studies found cured onions ('*Spartan Banner 80'*) to be more sensitive to impact than those freshly harvested, with drop heights as small as 45 mm for cured bulbs,

and 65 mm for fresh bulbs resulting in a 100% probability of bruising (Timm et al. 1991). Bruising itself has not been reported as a primary reason for the rejection of Tasmanian onion bulbs from export markets (J Dennis 2014, pers Com). Instead, physiological changes such as sprout growth, weight loss and skin loss appear to be of greater concern.

Onion bulb storage and post-harvest handling have not received the same level of attention as other economically important fruit and vegetables, and challenges remain in ensuring the reliable supply of high quality, durable bulbs with long storage life to counter seasonal markets. In particular, there continues to be uncertainty on the effect of mechanical impact in reducing the storage quality of bulbs. Previous studies have used cultivars and production systems which differ from those used by the Tasmanian export industry. This thesis has been conducted to address the paucity of scientific knowledge in these areas to develop and refine management strategies that improve the reliable supply of high quality, durable bulbs with long storage lives.

Thesis scope

This study aimed to address challenges associated with reliably producing and supplying high quality bulbs to export markets. From direct consultations with the onion industry of Tasmania and a revision of the scientific literature, three major areas of investigation for improving the reliability and consistency of ‘Class One’ bulb supply to Europe were identified. These areas included: 1) The effect of post-harvest mechanical impacts on bulb storage; 2) determining the optimal time to interrupt plant growth and development when

lifting plants from the soil and; 3) describing the mechanisms that regulate the plant development marker, tops-down.

In Chapter 2, the effect of mechanical impacts on metabolic activity and storage of onions is examined in controlled impact and packing line experiments. Results from the study will enable the industry to make informed decisions when balancing the trade-offs between handling efficiency and storage potential. There is little understanding of the extent Tasmanian industry handling systems affect bulb storage potential.

In Chapter 3, the influence of pre-harvest factors including timing of lifting, nitrogen fertiliser application and defoliation treatments on the transition from active bulb growth to dormancy are reported. Links between pre-harvest growth and development and the post-harvest bulb storage quality are highlighted and discussed.

Chapter 4 investigates the relationship between changes in pseudostem properties and canopy collapse. Despite the importance of pseudostem lodging to the industry practice of lifting bulbs, only qualitative descriptions have been previously published. A better understanding of the link between bulb development and pseudostem failure may improve the scheduling of crop lifting.

Building on the understanding of pseudostem properties gained in Chapter 4, Chapter 5 explores the softening of tissue at the pseudostem base among cultivars as an alternative

marker for crop development. It also investigates the relative role of tissue mechanical properties and water relations as the basis of the tissue softening process.

Chapter 6 provides a general discussion of the findings from Chapter 2, 3, 4 and 5 in the light of the existing literature and practical recommendations for the benefit of the Tasmanian onion industry.

Chapter 2: Onion (Allium cepa L.) bulbs are most sensitive to base plate impacts[1]

Abstract

Although onion bulbs have one of the highest storage potentials under ambient conditions of any vegetable, physical impacts to bulbs during post-harvest handling can reduce this capacity. This presents a challenge in production systems that rely on mechanised harvesting and handling operations to remain economically competitive. In this study, the respiration rate of onions sampled immediately after travelling over a commercial packing and grading line was found to be elevated compared with pre-grading samples. Elevated respiration rate was linked to a higher rate of weight loss and reduced storage potential. Controlled impacts applied to individual bulbs also increased the rate of bulb respiration and internal sprout growth. Bulbs were more sensitive to impacts to the base plate than to impacts to the equator or neck. Environmental scanning electron microscopy revealed intra-cellular disruption within the scales where bulbs were impacted on the equator. No cellular damage could be distinguished in bulbs impacted on the base plate. Minimising post-harvest respiration rate of mature bulbs is recommended for long-term storage. Furthermore, it is recommended that the design of handling equipment and assessment of bulb impact sensitivity consider both impact magnitude and position.

[1]Elements of this chapter were presented at the International Horticultural Congress 2014 and have been submitted for inclusion in Acta Horticulturae as published symposium proceedings.

Introduction

Vibration, compression, friction and impact forces during post-harvest handling operations can all reduce the storage life of fresh fruit and vegetables (Wills et al. 2007). These forces have been reported to damage plant tissue (Menesatti et al. 2001; Mitsuhashi-Gonzalez et al. 2010), increase metabolic rate (Burton et al. 1985; Saltveit 2000) and increase the susceptibility of produce to microbial infection (Eskin & Robinson 2010). While harvesting, sorting and packing of onions by hand can be used to minimise impact damage, this is not economically feasible in the low-cost production systems of regions where labour costs are high relative to market price.

In onions, physical impacts from vertical drops leading to physical injury such as bruising and pitting are considered a major concern during handling operations. In general, post-harvest handling systems are designed to limit or eliminate visible physical damage from sources such as vertical drops; however less consideration has been given to the physiological response of onions that receive impacts below the threshold for visible injury. In particular, few studies have investigated the relationship between mechanical impacts and onion bulb storage life. Herold et al. (1998) assessed the respiration response and storage loss of onion bulbs (‘*Rijnsburger Balstora’*) subjected to a range of mechanical loads by dropping them onto a conveyor belt or steel plate. These mechanical loads increased respiration rate both immediately after impact and over the subsequent

11-week storage period. The authors also reported a positive relationship between drop height on to a steel plate and loss of bulb quality due to sprouting. In a separate study, cured onions (‘*Spartan Banner 80’*) were found to have larger bruises after impact than freshly harvested onions (Timm et al. 1991). Thus curing of the leaf sheaths appear to play a role in increasing the sensitivity of onions to physical impact. In the latter study, the authors did not measure storage life of the bulbs.

A range of methods have been used to simulate the mechanical impacts of commercial handling operations for various fruit and vegetables including dead drops onto a control surface (Herold et al. 1998), pendulum mediated impacts (Van linden et al. 2006) and, objects of a specific mass dropped from a set height onto the produce (Sparrow et al. 2003). The latter technique applies impacts of a known force to a specific position, in contrast to the ‘dead drop’ method where, force varies with the mass of the object and, the position of impact is often left uncontrolled.

Non-visual responses of fruit and vegetables to mechanical impacts include metabolic and biochemical changes such as increased biosynthesis of ethylene leading to increased rate of ripening (Moretti et al. 1998) and elevated metabolism (Burton & Schilte-Pason 1985; Saltveit 2000). The rate of metabolic activity has been shown to be an important determinant of storage life of produce (Wills et al. 2007). As such, the measurement of respiration rate has therefore been a common practice for estimating metabolic activity in post-harvest physiological studies, though the link between respiration rate and storage potential is not always well defined. For example, Yasin et al. (2007) found emergence

from bulb dormancy was associated with increased respiration rate in stored bulbs. Benkeblia et al. (2000) showed a positive relationship between respiration rate and ionising radiation treatment. Ward et al. (1976) found that exogenous applications of the sprouting inhibitor, maleic hydrazide, prevented the increase in respiration rate seen in untreated bulbs during long term storage.

In the market place, the appearance of sprout leaves indicates the end of a bulbs storage life and loss of market acceptance. Studies that investigate storage life therefore measure the time to visible sprout emergence, the beginning of sprout growth, or the extent of internal sprout growth (Abdalla & Mann 1963; Brewster 1987; Chope 2006; Pak et al. 1995; Yasin & Bufler 2007). Measuring internal sprout growth would ideally provide a predictive estimate for sprout emergence and the completion of storage life. A range of techniques for measuring internal sprout growth have been employed for research purposes, but there has been no consensus on the optimal method for measuring sprout growth. A challenge in measuring internal sprout growth is the delineation of sprout tissue from scale tissue. Some authors have used the presence of green pigmentation to delineate sprout from scale layers (Chope 2006). However, there is little evidence that all developing sprout tissue contains green pigment during early development across cultivars. Pak et al. (1995) reported a linear sprout growth rate for ‘Hysam’ bulbs, however the differentiation of sprout tissue lacked clarity and the selected cultivar appeared to have a short or no dormancy period. Yasin and Bufler (2007) used a change in blade length within a sprout size range from a sample population to differentiate bulbs which had initiated sprout growth but did not use this parameter to monitor the

progression of sprout growth. Based on detailed morphological observations of Heath et al. (1965), Brewster (1987) used a leaf ratio (blade : sheath) equal to or greater than 0.25 as a well-defined, repeatable means for distinguishing the transition from fleshy scale tissue to sprout tissue. This would appear to be the most reliable method for the measurement of internal sprout growth when assessing a response to mechanical impacts.

While harvesting, sorting and packing of onions by hand can be used to minimise impact damage, this is not economically feasible in the low-cost production systems of the developed world, where labour costs are higher relative to market price. In industrialised systems, bulbs receive physical impacts of varying magnitudes and frequencies during mechanical lifting, harvest, handling and grading operations. Assessments of the mechanical impacts during the handling and grading processes of the facility used in this study have been undertaken using an instrumented sphere fitted with pressure sensors. The highest impact forces occurred at the removal of pseudostem and root material using a ‘scroll topper’ and, the sizing of bulbs using screens (McPhee 1998).

A greater understanding of the effects of the physical impacts on onion bulb metabolic activity and storage life would assist supply chain participants in making informed decisions with respect to handling practices. This paper assessed the effects of mechanical impacts on bulb respiration and storage life of onions that had either been: 1) processed in a commercial packing facility or; 2) subjected to a range of controlled simulated mechanical damage. We hypothesised that mechanical impacts lead to elevated respiration rates and reduced storage life dependent on the location and magnitude of

impact. Further, these impacts may not be visually evident, with pitting and bruising absent, and damage not being seen externally until sprouts show through the neck earlier than bulbs not subject to mechanical impact.

Materials and methods

The effects of mechanical impact on bulb respiration rate and sprout growth were investigated using bulbs from commercial crops grown in Northern Tasmania, Australia. Experiments were designed to assess the short- and long-term responses of onions subjected to: A) commercial grading and packing; B) controlled impacts of varying magnitude and position; and C) controlled impacts followed by simulated storage and handling conditions. Cultivars were maintained consistent within each experiment but varied among experiments due to availability. All bulbs were then stored at 19°C and 65% relative humidity unless stated otherwise

Commercial packing line assessment

Bulbs (‘*Regular Creamgold’)* were sourced from three commercial crops (CGa11, CGb11 and CGc11) following mechanical harvesting in the 2010/11 season. The packing line which was assessed utilised bulk unloading of onions from containers or bunkers, transfer belts, inspection belts, scroll toppers where the roots and neck are removed, size grading plates, and further visual inspection before loading into bulk containers or bags. For each crop, four or five replicate samples (8-10 kg each) were collected at three locations along the packing line: 1) prior to the removal of remaining pseudostem and root material by a

scroll topper (pre-topping); 2) immediately after the removal of pseudostem and root material by a 'scroll topper' (post-topping), and; 3) immediately after size grading by shaking screens (post-grading). Bulb respiration rate and weight of each replicate was measured one day after collection, then weekly and later monthly. The number of bulbs that sprouted or disintegrated was recorded at regular intervals and discarded. Measurements were taken over a period from 1 day after sampling up to the end of the monitoring period (277 to 303 days) after collection from the packing line. Recorded mass loss was inclusive of discarded bulbs.

Controlled impact

Two controlled impact experiments were undertaken to assess the effects of: impact position and; magnitude of impact on individual bulb respiration and sprout growth under controlled storage conditions and one to assess the location of impact with simulated shipping and handling. All controlled impact experiments used a 130 g steel sphere dropped down a vertically orientated tube (40 mm in diameter) onto an onion held against the base of the tube.

Impact position - constant storage conditions

Impact treatments were applied to onion bulbs ('*Plutonus*') from a 90 cm drop height, with five replicate bulbs used for each treatment in the 2013 season. The treatments were: 1) untreated control; (C treatment); 2) impact to the base plate (B treatment); 3) impact to the equator (E treatment) and; 4) impact to the top of the bulb (T treatment). The

respiration rate (described below) of individual bulbs was measured before impact and immediately afterwards, then at 2, 5, 9, 20, 32, 45, 69, 142, 192, 263, 311, 408, 578 hours after impact. Internal sprout growth assessments (described below) were carried out on every bulb 100 days after treatments were applied. Structural changes at the cellular level were examined in additional bulbs that had been treated with the 90 cm drop plate either on the base plate or equator using environmental scanning electron microscopy (ESEM) at magnifications of 100X to 300X at 5Kv to 20KV(FEI Quanta MLA 600, Hillsboro, Oregon, USA).

Impact position -simulated storage and handling

Bulbs were sampled from two commercial crops (*'Regular Creamgold'*) at the intake of a commercial packing line. Each replicate sample comprised 10-11 kg of bulbs, which were stored in net bags. Each bag was weighed prior to application of treatments and was randomly allocated to one of four replicates (12 samples per crop) to receive either Control (C), Base (B) or Equator (E) treatment as described above. Bulbs were then stored for 12 hours at 24 °C and 12 hours at 14 °C to simulate diurnal temperature changes typical of ambient storage. After 100 days in storage, bags were weighed to determine weight loss, then tumbled in a rotating plastic drum for 5 minutes to provide a consistent skin retention test, this based on a modification of the method used by Gracie (2006). The bulbs were segregated into skin fault (all bulbs with visible flesh layers) or no skin fault before being counted and weighed. Bulbs were then transported to a laboratory and 10 bulbs with skin faults and 10 bulbs without skin faults from each treatment

replicate were assessed using the internal sprout growth assessment method described below.

Impact magnitude - constant storage conditions

The effect of the magnitude of impact applied to the base plate of bulbs (‘*Early Creamgold’)* was examined by dropping a steel ball from 0 (control), 30, 90 or 150 cm directly above the bulb in the 2014 season. Respiration rate was measured before and immediately after impact, then at 4.5, 24, 49, 96, 336, 672 hours after impact. Internal sprout growth assessments were carried out on all bulbs at 100 days after impact.

Respiration rate

Respiration rate was measured by placing bulbs in sealed containers (18000 and 500 cm^3 for packing line assessment and controlled impact studies, respectively) to create a closed system configuration. In all measurements the input and output tubes of the containers were place opposite each other to ensure air flow across the bulb(s). The CO_2 concentration of the air from the containers was analysed with an infrared red gas analyser (ADC 2250, ADC BioScientific Ltd, UK). The concentration of CO_2 was logged over a 3-10 min period and the linear rate of change was used to calculate respiration rate and expressed in mmol CO_2 kg^{-1} h^{-1}. For the impact magnitude experiment, bulbs were sealed in 500 cm^3 plastic containers and left to equilibrate for at least 6 min before CO_2 was measured with an infrared red gas analyser (LI-6400XT, LI-COR, USA). A sample cell outlet manifold kit was installed to isolate the sample cell from the LiCor 6400xt leaf

chamber which allowed it to be used as a stand-alone gas analyser. Measurements were made at an ambient CO_2 partial pressure of 400 μmol CO_2 mol^{-1} at 19 °C in an open system configuration.

Internal sprout growth assessment

The weight, height and width of individual bulbs were recorded prior to assessment. Height was measured from the base plate to the neck base while width was measured at the equator. The bulbs were then dissected to separate all sprout tissue, defined in this study as leaves with a blade to sheath ratio of greater than 0.25 (Brewster, 1987). The total fresh mass, number of sprouts and length of the longest sprout per bulb were recorded. All length, height and length measurements were carried out using Vernier callipers.

Statistical analysis

Statistical analyses were performed using SPSS (ver. 22). Data were analysed using a general linear model or mixed model, depending on the experiment. Assumptions of normality and homoscedasticity were assessed and data transformed where necessary to meet these assumptions. *Post hoc* tests were computed using Fishers Protected Least Significant Difference (LSD).

A mixed model was used to test the sampling point along on the grading line, which included crop, sample position and crop by sample position interaction as fixed factors. Sampling time within crop was used as random effect to test crop effect and the residual was used to test sample point and sample point by crop.

A general linear model was used to analyse sprout length and mass data from the controlled impact and simulated handling and grading experiment. Crop and impact treatment effects were included as fixed factors and tested against the random term of replicate within crop by impact position. Skin category (with and without faults) was treated as nested within replicate and tested along with interactions with the main (crop and impact) effects tested against the residual error.

Results

Commercial packing line assessment

There were significant differences in respiration rates among crops at the intake point (pre-topping) ($F_{2,12}$ = 27.3; p<0.001). Specifically, there was a two-fold difference between the lowest rate observed for crop CGa11 (0.095 m mol CO_2 kg^{-1} hr^{-1}) and crop CGc11 which had the highest rate of 0.205 m mol CO_2 kg^{-1} hr^{-1} (**Figure 2-1**).

One day after sampling, the mean respiration rate of bulbs sampled post-topping and post-grading was higher ($F_{2,22}$ = 22.0; p<0.001) than bulbs sampled pre-topping in all three crops. The interaction between onion sampling position by crop were not significant ($F_{3,21}$ = 0.49; p = 0.693) (**Figure 2-1**).

Following the initial peak at one day after sampling, respiration rates declined to minimum values between 25 and 40 days in storage for all crops. In particular, respiration rates were the lowest for crop CGa11 (0.04 m mol CO_2 kg^{-1} hr^{-1}) followed by crop CGb11 0.08 m mol CO_2 kg^{-1} hr^{-1} and CGc11 0.09 m mol CO_2 kg^{-1} hr^{-1}.

Following this initial drop in respiration rate, mean bulb respiration rate increased slowly over the remainder of the storage period for all sampling points and crops. When respiration was considered over the long-term, respiration rate was higher in bulbs sampled post-topping and post-grading than pre-topping samples for crops CGb11 CGc11 but not for CGa11 (**Figure 2-2**).

The proportion of bulbs (by number) that were discarded due to internal breakdown and/or externally visible sprouting was significantly higher ($F_{2,31}$=4.12; p=0.027) in post-topping and post-grading than pre-topping samples (**Table 2-1**). No difference in these attributes was detected between post-grading and post-topping, and no interaction between crop and sampling position ($F_{3,21}$ = 2.86; p=0.061) was recorded.

Total bulb loss during storage (277-303 days) was significantly affected by crop ($F_{2,29}$ = 27.44; p<0.001), sampling position ($F_{2,29}$ = 8.29; p=0.001) and their interaction ($F_{2,29}$ = 3.03; p=0.045). Specifically, crop CGa11 did not lose additional bulbs post-grading in contrast to onions from crops CGb11 and CGc11. Overall, bulb loss during storage from crops CGb11 and CGc11 was approximately double that of crop CGa11 (**Table 2-1**), and bulbs sampled post-topping and post-grading were generally higher than pre-topping.

Table 2-1. Weight loss and proportion (by number) of bulbs discarded following long-term storage. Bulbs were sampled from a commercial packing line: prior to topping (pre-topping), after travelling over a scroll topper (post-topping), and size grading screens (post-grading). Values are means ± 1 SEM, n=5. No post topping samples were collected for crop CGa11. Weight loss is inclusive of discarded bulbs.

Crop	Days after sampling	Sampling position	Bulb loss (% mass)	# Bulbs discarded (%)
CGa11	303	pre-topping	18.1 ± 1.2	4.0 ± 1.0
		post-grading	20.2 ± 1.0	3.7 ± 1.4
CGb11	263	pre-topping	33.8 ± 3.9	26.1 ± 4.7
		post-topping	41.3 ± 3.5	33.8 ± 3.2
		post-grading	53.5 ± 5.5	44.8 ± 6.3
CGc11	277	pre-topping	30.7 ± 2.4	22.3 ± 2.6
		post-topping	42.7 ± 6.3	31.5 ± 5.3
		post-grading	41.3 ± 2.4	29.4 ± 2.7

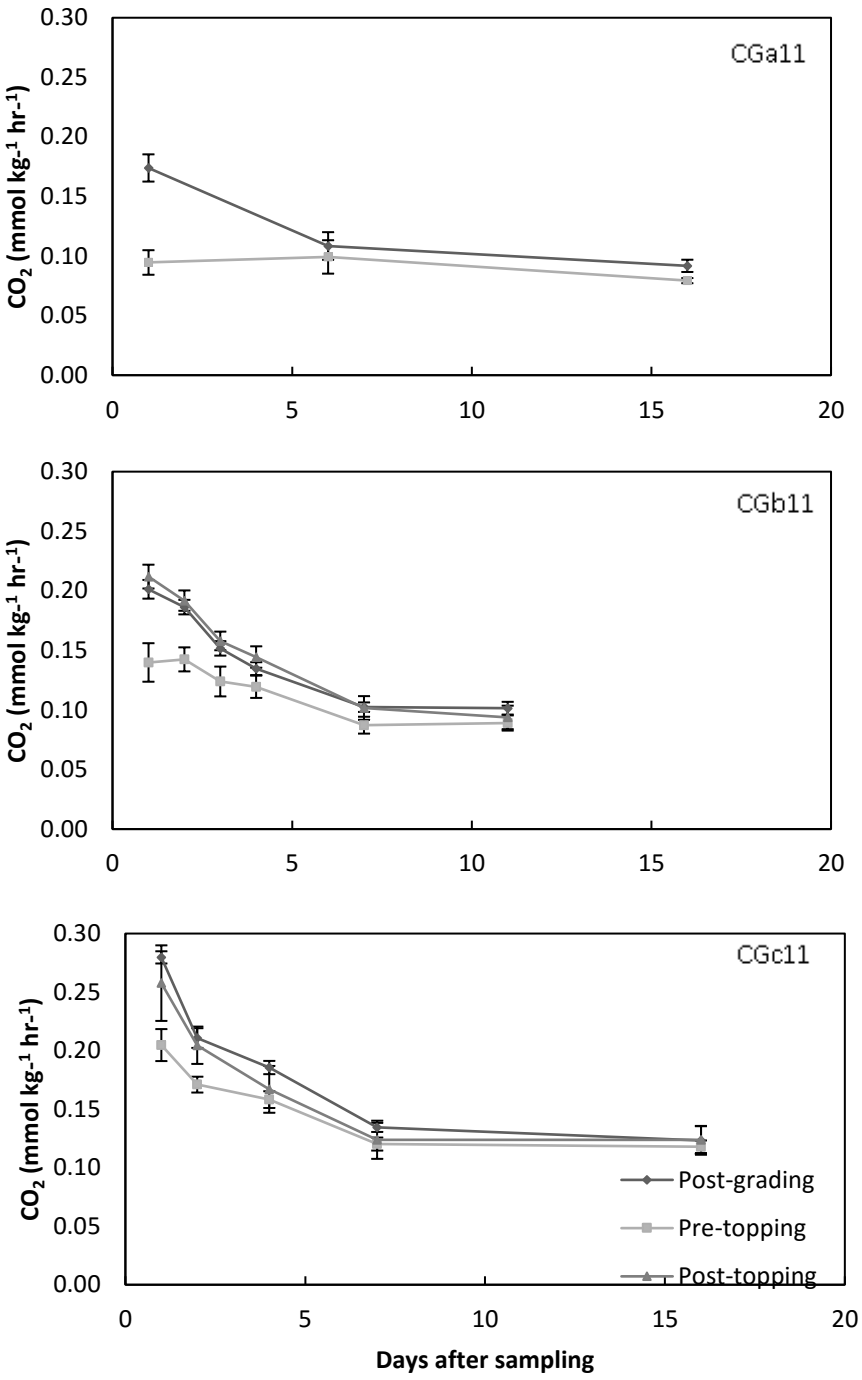

Figure 2-1 Mean respiration rates of bulbs over 16 days after sampling from three crops (CGa11, CGb11, and CGc11) while being graded and packed on a commercial packing line. Onions were sampled prior to topping (pre-topping), after travelling over a scroll topper (post-topping), and after travelling over shaking size grading screens (post-grading). Error bars indicate ± 1 SEM, n= 4 or 5.

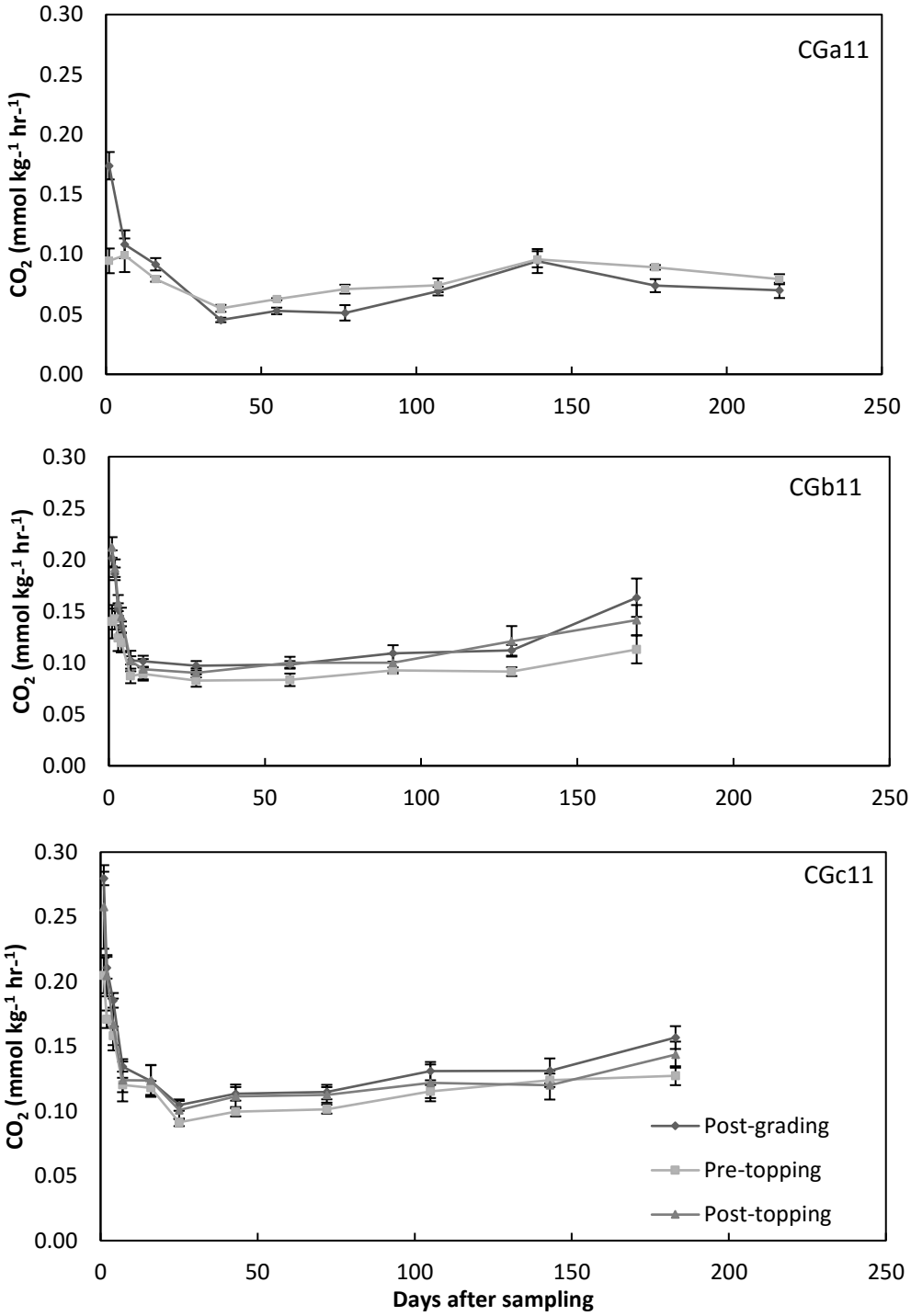

Figure 2-2. Mean respiration rates of bulbs over 169-217 days after sampling from three crops (CGa11, CGb11, and CGc11) while being graded and packed on a commercial packing line. Onions were sampled prior to topping (pre-topping), after travelling over a scroll topper (post-topping), and over size grading screens (post-grading). Error bars indicate ± 1 SEM, n=4 or 5.

Impact position - constant storage conditions

Bulb respiration rate peaked approximately 32 hours after physical impact to all position treatments (**Figure 2-3**). Bulbs in the base plate (B) treatment had a higher peak respiration rate ($F_{2,15} = 7.57$; $p = 0.007$) than all other treatments (**Figure 2-3**). Respiration rate was similar between impacts to the bulb equator (E) and bulb top (T) treatments. After this initial increase in treated bulb respiration, there was a common decline in respiration rate over the following week; however those in the base plate impact treatment maintained a higher respiration rate at each assessment time. Consistent with the higher level of respiration, bulbs in the base plate treatment had significantly longer and heavier sprouts as a proportion of bulb height ($F_{2,15} = 12.50$; $p = 0.001$) and mass ($F_{2,15} = 8.54$; $p = 0.005$), respectively, than the other treatments at 100 days post impact (**Figure 2-4**).

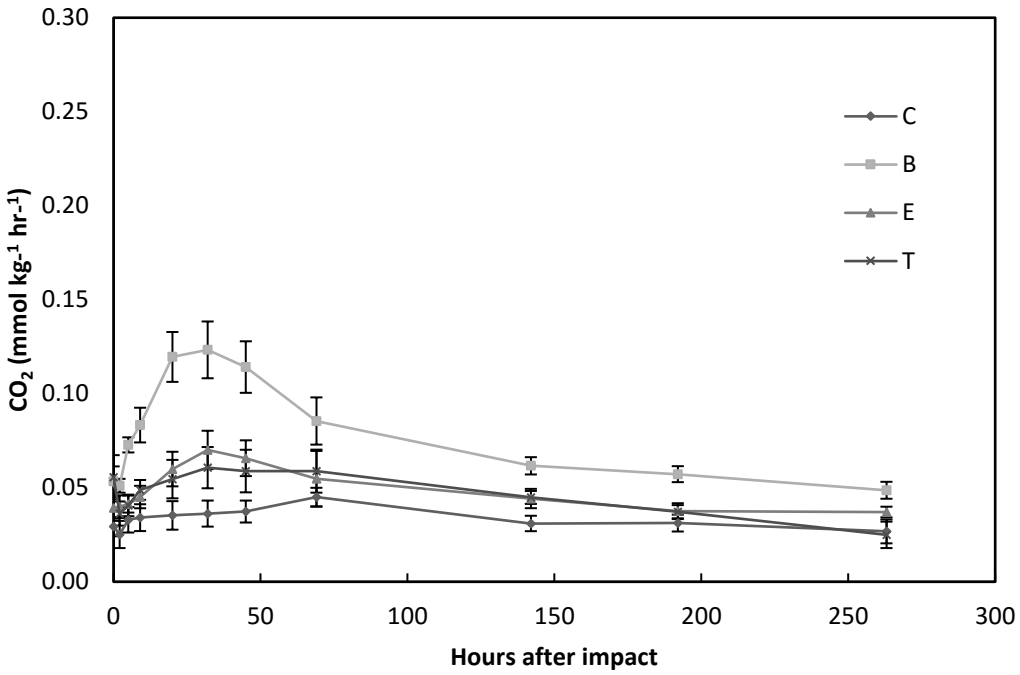

Figure 2-3 Mean respiration rates of bulbs that were impacted at t=0, at different positions on the bulb surface; Base (B), Control (C), Equator (E), Top (T), with a 130g steel sphere from a height of 90cm. Error bars indicate ± 1 SEM, n=5.

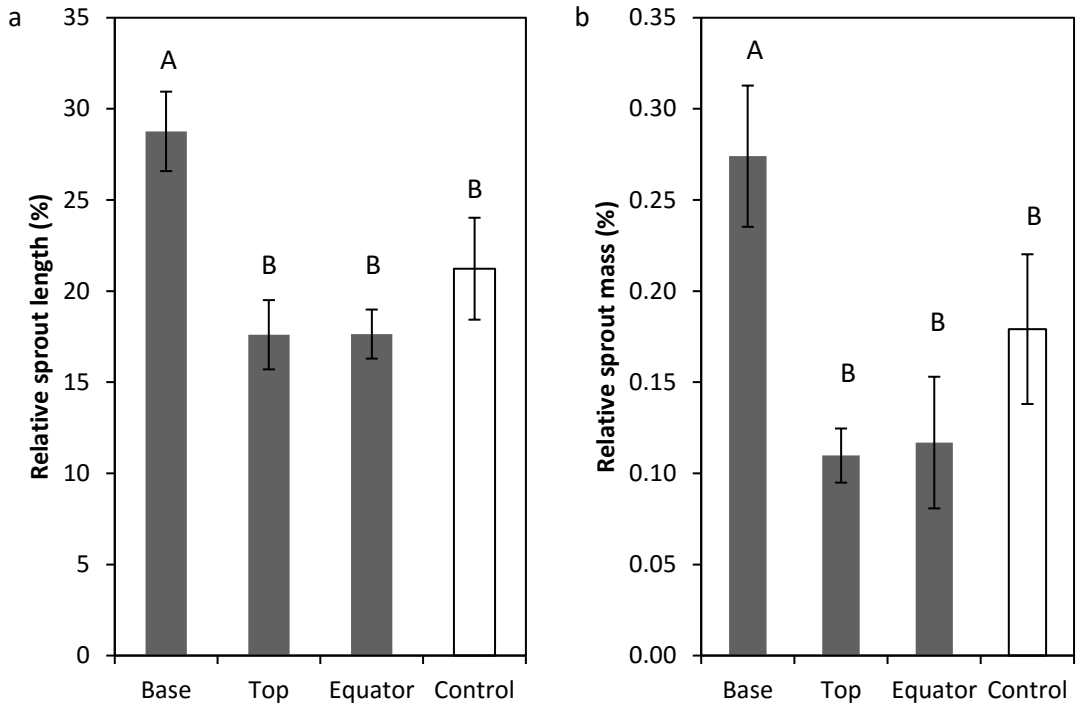

Figure 2-4 Mean (a) sprout length as a proportion of the bulb height and (b) sprout mass as a proportion of bulb mass 100 days after controlled impact to Base, Equator, Top or no impact (Control) from 90 cm with a 130 g steel sphere. Error bars indicate ± 1 SEM, n=5. Different letters indicate significant differences among treatments using Fishers LSD (5%).

The ESEM analysis revealed that bulbs in the equator (E) treatment had fractures in the first and second outermost fleshy tissue layers (**Plate 2-1** (EI)). These fractures were orientated tangentially and vertically. The outer scale in Plate 2-1 (EI) appears to have separated tangentially from the interface with the lower scale, and there is some evidence that may indicate cell wall tearing. Damage to the second scale is radial in nature, appearing to extend throughout the majority of the tissue. No fractures were observed in the third fleshy scale of the same bulbs. There was no visible evidence of tissue damage in bulbs in the B treatment (**Plate 2-1** (BI)).

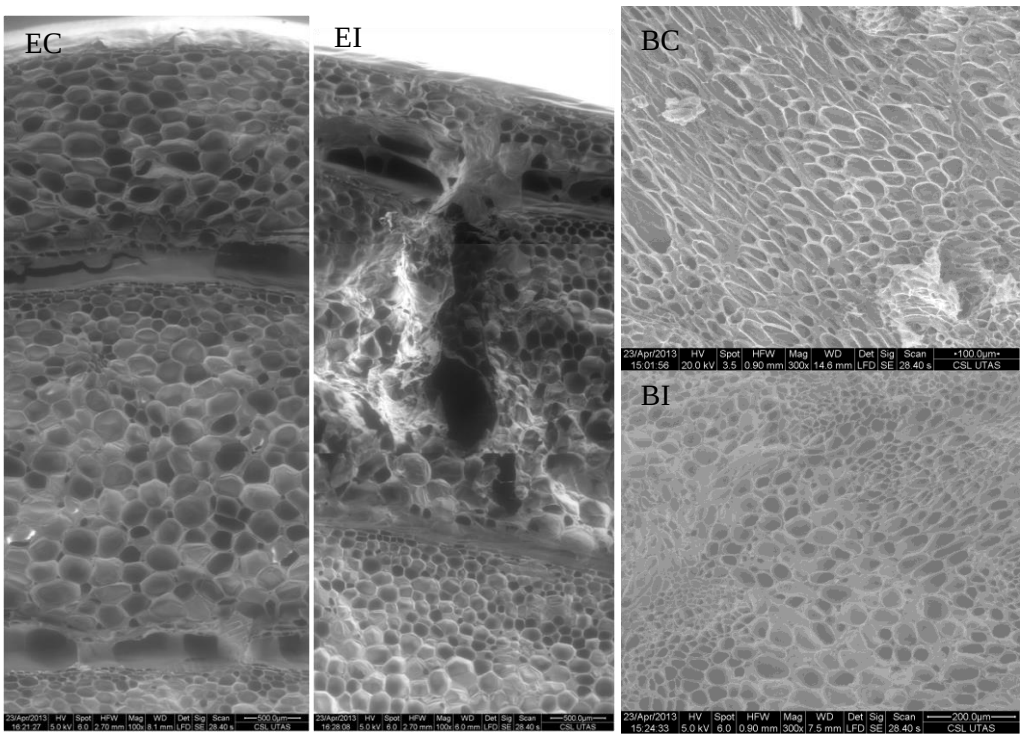

Plate 2-1 Environmental electron scanning microscopy images of onion tissue directly below impact site. Images are from (EC) Equator Untreated Control, (EI) Equator Impacted, (BC) Base Untreated Control, and (BI) Base Impacted.

Impact position - handling and storage simulation

Bulbs in the base impacted treatment and subjected to 100 days storage and simulated handling had a higher proportion of skin faults, 76.1%, compared with 66.8% for the untreated control and 69.6% for those in the equator impacted treatment ($F_{2,18}$ = 8.69; p=0.002). The proportion of bulbs with skin faults was higher in crop CGb13 at 82.3% than in crop CGc13 at 59.4% ($F_{1,18}$ = 149.9; p<0.001). Furthermore, bulbs with skin faults had longer and heavier sprouts than those without skin faults (**Figure 2-5**And **Table 2-2**). An interaction between skin category and impact treatment was recorded for sprout length ($F_{2,18}$=8.0; p=0.003) (**Table 2-2**).

Sprout growth in bulbs from CGb13 was higher than CGc13 across all parameters but only length was statistically significant (**Table 2-2**). No significant differences were detected among impact treatments however there was a trend of a higher sprout growth in base impacted bulbs (**Table 2-2**). No interaction between impact by position crop was recorded.

Table 2-2 Effect of crop and physical impact position on onion sprout number, and sprout length and fresh weight as a proportion of bulb height and mass, respectively. Bulbs were segregated into two groups: with and without skin faults. The main effects were tested against the main effect error term, while the nested skin term and its interaction with the main effects were tested against the residual error.

			Number		**Length**		**Mass**	
Fixed Effects	**Num. d.f.**	**Den. d.f.**	**F Value**	**Sig.**	**F Value**	**Sig.**	**F Value**	**Sig.**
Crop	1	18	0.337	0.569	3.988	0.061	0.393	0.538
Impact	2	18	0.053	0.948	4.267	**0.030**	1.360	0.282
Crop *Impact	2	18	0.385	0.686	0.739	0.492	0.346	0.712
Skin	1	18	1.610	0.221	84.511	**<0.001**	60.377	**0.001**
Skin*Treat	2	18	0.346	0.712	7.969	**0.003**	1.366	0.280
Skin*Crop	1	18	3.909	0.064	1.285	0.272	0.001	0.977
Skin*Treat*Crop	2	18	0.377	0.691	1.366	0.280	0.342	0.715

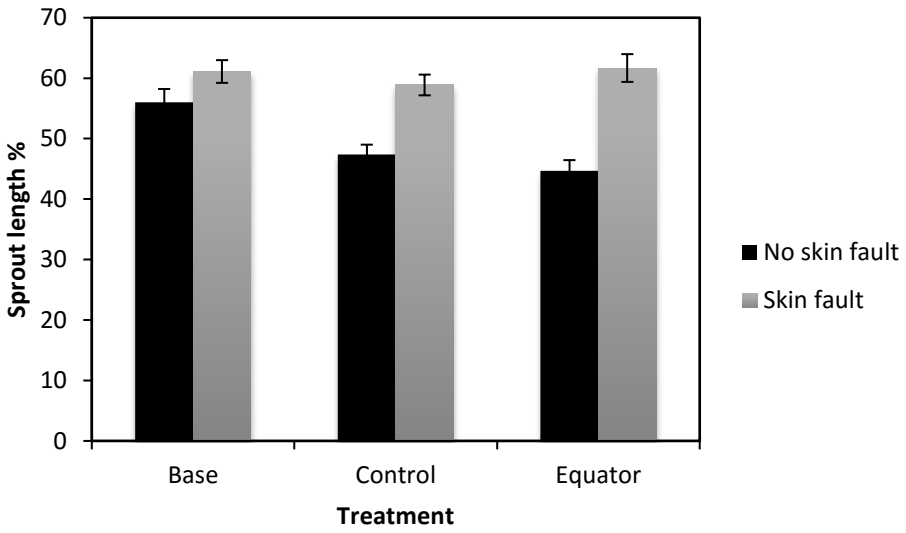

Figure 2-5. Mean sprout length as a proportion of the bulb height 100 days after controlled impact to Base, Equator or no impact (Control) from 90cm with a 130g steel sphere. Error bars indicate ± 1 SEM, n=5.

Impact magnitude - constant storage conditions

There was a positive relationship between magnitude of impact and subsequent peak bulb respiration rate (Spearman's r = 0.94, $p<0.005$), with respiration rate reaching levels more than three-fold that of the untreated controls (**Figure 2-6**). For bulbs that received a single impact, sprout length percentage was greater in bulbs struck from 150 cm ($F_{3,25} = 3.98$; $p=0.019$) than those treated at lesser magnitudes 100 days post-treatment (**Figure 2-7**). A similar trend was observed with the second impact treatment, however six out of the eight bulbs that were impacted a second time from a 150 cm drop height showed internal breakdown of the inner scales adjacent to the sprout within the 100 day storage period (**Figure 2-8**). This second impact possibly caused by secondary infection by a pathogen (**Plate 2-1**). Only one internal breakdown was observed for bulbs impacted a second time from lower drop heights.

Plate 2-1. Onion bulb showing internal breakdown 100 days after initial impact after being struck on two occasions 28 days apart by a 130g steel sphere dropped onto the bulb from 150cm.

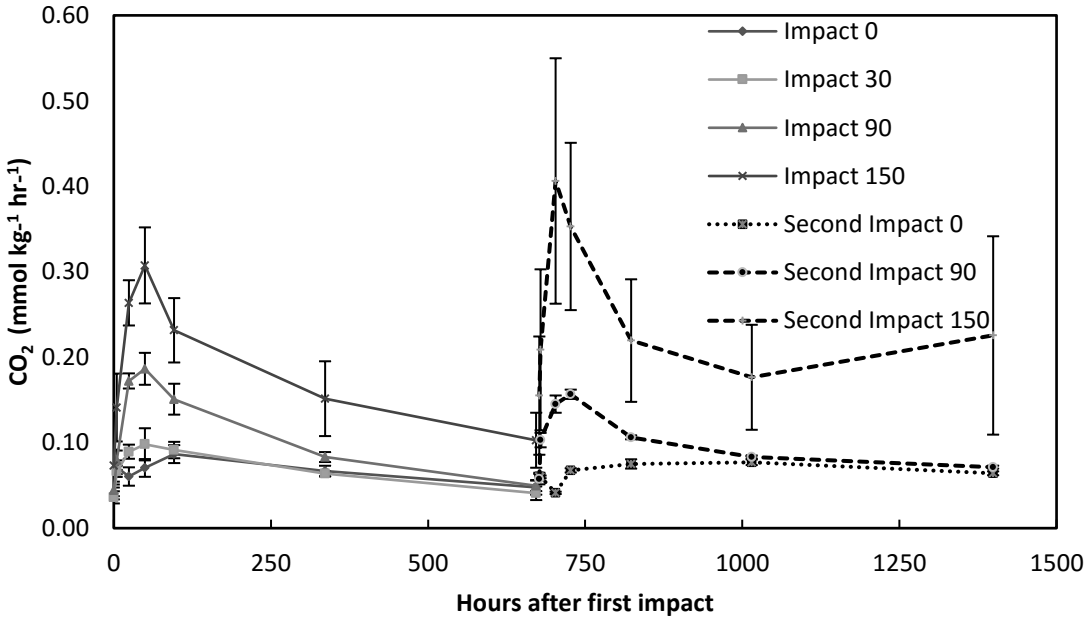

Figure 2-6 Respiration rates of bulbs impacted to the base plate by a 130 g steel ball dropped vertically from 0 (control), 30, 90 or 150 cm height above the bulb, and re-impacted (Second Impact) 28 days later from the same height. Error bars indicate ± 1 SEM, n=4.

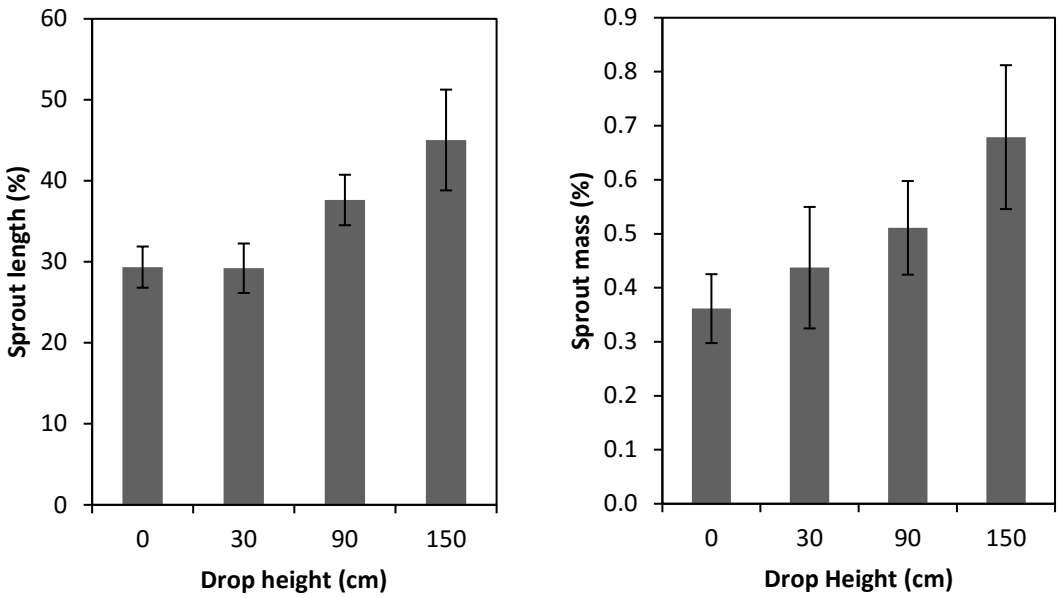

Figure 2-7 Sprout growth assessment 100 days post impact with a 130 g steel sphere from 0 (control), 30, 90 and 150 cm , sprout length percentage (a), Sprout mass percentage (b). Error bars indicate ± 1 SEM.

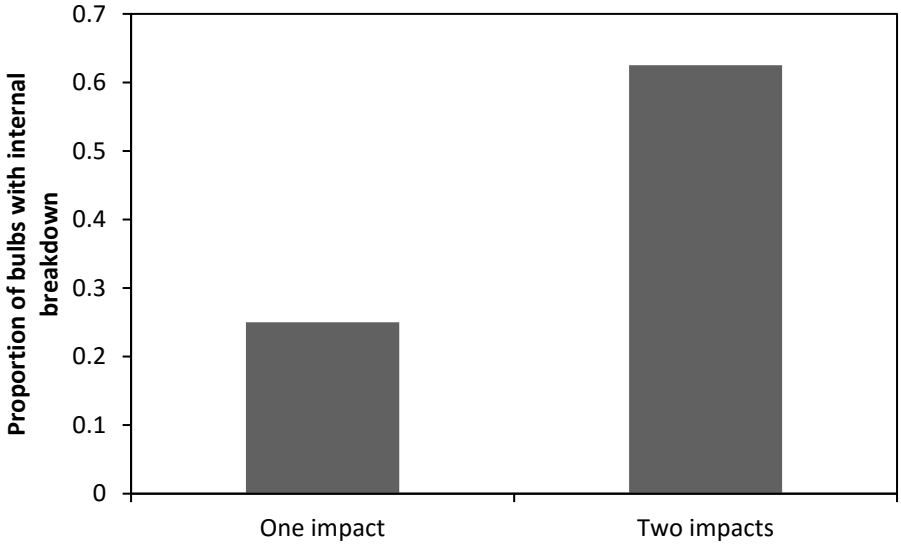

Figure 2-8. Proportion of bulbs with internal breakdown after 100 days of storage. Impacted with a 130 g steel sphere from 150 cm drop height. One impact at day 0 or two impacts; day 0 and day 28.

Discussion

In this study, controlled mechanical impacts increased respiration rates and reduced shelf life and quality, as evidenced by increased sprout growth and skin faults, particularly when impacts occurred at the base plate. In contrast to earlier studies that did not take into account the position of impact, these results suggest that the location of impact is an important consideration for bulb storage life. Findings from this study did confirm previous observations that reduced bulb quality is related to increasing magnitudes of impact and the occurrence of secondary impacts. In both these instances, greater respiration rates and increased sprout growth associated with the high or secondary impact treatments.

Respiration in living tissues can be classed as either growth respiration, or maintenance respiration, with the latter playing a predominant role during dormancy or resting phases (Amthor 1984). Respiration after lifting declines during curing and often into the early storage period (Gracie et al. 2009), and subsequently increases as dormancy breaks and sprout growth occurs (Yasin & Bufler 2007). In this study, the controlled impact experiments demonstrated that physical disturbance can disrupt this pattern, increasing respiration rates, which afterwards do fall, but not back to rates that were initially observed. The greater increase in respiration rate and sprout growth recorded after base impact treatments may be due to stimulation of the meristematic zone, or part of a wounding response to trauma. Certainly, increased respiration in sprout tissue has been

observed in this zone when bulbs have emerged from dormancy (Yasin & Bufler 2007) and it is possible that the increased respiration rates observed were the result of a comparatively earlier break in dormancy, as evidenced by larger sprouts after 100 days of storage following impact to the base plate. The ESEM images indicate clear differences in the nature of the tissues between base and equator treatments. While extensive damage was observed in equatorial tissues, very little physical damage was observed in tissues of the true stem. This could be due to differences in tissue rheological properties between the fleshy layers at the equator and the dense compressed stem (base plate), with the latter exhibiting greater viscoelastic behaviour under impact. There may also be difference in the biochemical wound response such as the release of Ethylene, which may warrant further investigation.

In all experiments, despite the differences in cultivars, respiration rates followed a similar pattern of an elevation in respiration rate in the 1-2 days post impact, followed by a reduction in respiration rate over the following 5-10 days, before increasing gradually over the following 250+ days. The initial decline after impact is consistent with the respiration pattern observed by Herold et al. (1998), however these investigators did not note a subsequent increase in respiration, possibly due to the shorter 77 day assessment period. Secondary impacts applied 28 days following an initial impact increased respiration rates, particularly from the 150 cm drop height, which caused a greater increase and variability in respiration rate. This result demonstrates that multiple handling events during storage and shipping can have a cumulative effect in elevating bulb

respiration rates. Similarly, Herold et al. (1998) found comparable peak respiration rates amongst bulbs treated with the same cumulative drop height, but higher respiration rates were subsequently maintained when fewer impacts of a greater magnitude were applied. This increase in respiration later during bulb storage is also consistent with observations that bruising sensitivity also increases after two months storage (Timm et al. 1991). While we have provide evidence bulbs may be more sensitive to secondary impacts, to confirm this, a comparison against bulb that were only impacted in the second round of impacts is needed.

Skin quality is a key attribute in market acceptance, and bulbs must retain at least one intact skin to cover the fleshy layer beneath. In this study, in addition to increased respiration rates and shorter storage life, greater sprout length was also associated with increased skin faults in impacted bulbs, with this particularly evident in those bulbs impacted at the equator, or the untreated control. This phenomenon may be related to the changes in bulb shape and associated fracturing of the skins at the base plate as the bulbs break dormancy (Tanaka et al. 1985).

In this study, crop as a factor significantly influenced sensitivity to impacts and vibration after travelling across the same commercial packing line and post-harvest storage life. Even though all three crops were grown in the same region under similar agronomic regimes, they exhibited different respiration rates prior to treatment. Previous studies have attributed differences in post-harvest storage to weather conditions (Suojala 2001), crop

nutrition (Wright 1993) and plant maturity at lifting (Suojala 2001; Wright 1993). It is unclear which pre-harvest factors differed among the crops studied here, or even if a different impact regime was experienced by the crops during harvesting and transport; however, a positive association between respiration rate prior to handling on the packing line and weight loss as well as sprouting and internal breakdown was observed. This illustrates that an understanding of the underlying respiration prior to grading and the effect of pre-harvest management may provide insights into the storage potential of crops.

Bulbs subjected to the commercial packing line had a shorter storage life and higher respiration rate than those sampled prior to topping and grading. This link between bulb handling and a reduction in storage life was most pronounced in bulbs from Crop CGc11 which had the highest pre-grading respiration rate. Therefore, bulbs may vary in their sensitivity to impacts and higher respiration rates may be an indicator of this sensitivity. The small difference in peak respiration rate of bulbs sampled post-topping and post-grading suggests that either the topping equipment had the greatest effect on bulb respiration rate, or that some upper limit to respiration was reached during the topping process. The data does demonstrate that the impacts bulbs received on the packing line had a significant effect on the metabolism of onion bulbs. This is further supported by the increase in bulb fresh mass loss and bulb deterioration due to sprouting and disease post topping and grading in crops CGb11 and CGc11. Again the question as to the importance of the pre-harvest environment and different management between crops is highlighted

and the role of these factors in determining post-harvest outcomes warrants further investigation.

The findings of this work indicate that in order to maximise bulb storage potential, bulb handling should minimise the magnitude of mechanical impacts, particularly to the base plate of bulbs. Additionally the differences in initial respiration rates between crops suggests that pre-harvest management or handling prior to packing of onion crops is pertinent to establishment of the base maintenance respiration rate, a bulbs sensitivity to impact, and a potential subsequent reduction in the storage life of crops.

Random orientation of bulbs during commercial handling operations may vary the location of impacts and therefore increase the variability in respiration rate and storage life among bulbs within a population. As current commercial packing lines do not control for the position of impact to bulbs it is recommended that packing line are designed and operated to both minimise the magnitude of impacts and avoid impacts to the base plate of bulbs. Future research on bulb sensitivity to physical impact should consider the effect of impact position.

Chapter 3: Interrupting onion plant development:

When to lift onion bulbs for maximum storage potential

Abstract

It is common practice in cool-temperate regions for commercial growers of onions to lift bulbs from the soil before crop growth, development, and skin curing are complete. Despite this practice there is limited understanding of the effect of time of lifting on the physiology of onion bulbs during long term storage. In this study, bulbs were lifted at scheduled intervals relative to when 80% of plant canopies were predicted to collapse within a crop, a developmental marker used commercially in Tasmania, Australia. This study was undertaken in five crops across three growing seasons. Delayed lifting resulted in higher yields, however lifting between 80 and 100% canopy collapse resulted in the lowest sprout length and sprout mass as a percentage of bulb weight and height, respectively, following long term storage. These findings support the recommendation that lifting onion crops at *ca* 90% canopy collapse maximises bulb storage life when grown in cool-temperate regions.

Introduction

Onion bulbs cultivated in cool-temperate climates are generally lifted out of the soil before leaf blade and pseudostem senescence is complete, or skins have cured. In low cost production systems such as in Tasmania, Australia, the lifted bulbs are windrowed and left on the ground for two to four weeks while the outer scales desiccate (cure) to form skins. In similar climates in parts of Europe, curing is undertaken artificially in facilities with circulated heated air for 3-6 weeks at 28C. In contrast, drier regions of other climate zones, leaf bales are allowed to senesce and bulbs cure in the ground prior to lifting (Currah et al. 1990). Thus time of lifting and curing method selected is predominately a function of the length and dryness of summer, and an interaction with plant physiology. In a review that examined time of lifting studies, Brewster (1990a) concluded that the optimal time for crop lifting was less than 100% tops down, which minimised time to sprouting and skin loss.

In cool-temperate regions the time of lifting of onion bulbs has generally been based on the proportion of plants within a crop that have developed to the stage of canopy collapse (Brewster 2008; Brewster et al. 1986; Gracie 2006; Komochi 1990; Mondal et al. 1986a; Nilsson 1980; Suojala 2001; Wright et al. 2001). This stage of development is sometimes referred to as “tops-down” or “top-down”(Wright et al. 2005). Mondal et al. (1986a) utilised the concept of 80% “tops-down” as a developmental marker for studies of crop phenology and referred to this point as “maturity date”. This choice of when to lift bulbs is based on reports that lifting at specific developmental stages affects yield (Sargent et al. 2001) and quality attributes such as number of intact outer skins (Wright et al. 2001), premature

sprouting (Komochi 1990) and incidence of rot (Wright et al., 2001). The decision of when to lift bulbs in cool-temperate climates is therefore generally considered a trade-off between total yield and quality.

While there is general support in the literature for lifting onions at 50 to 80% tops-down in cool-temperate regions, field studies in Tasmania show that the yield and quality responses to time of lifting can vary among sites and with seasons (Gracie 2006). In Finland, Suojala (2001) found that lifting could be delayed by as much as four weeks after 100% canopy collapse in dry years without increasing bulb storage losses during 4-8 months of controlled atmosphere storage, however there was a reduction in quality when lifting was delayed in wet years. In New Zealand, Wright (1993) recommended "late lifting" (>90% tops-down) in order to reduce field curing time, but later recommended 60-80% tops-down (Wright et al. 2001). These findings highlight the putative role of climate in determining the time of canopy collapse begins, and span of the optimal 'lifting window' for onion bulbs. The inability to predict crop responses to time of lifting may in part be due to a lack of understanding of bulb physiology as plants mature, and to the heterogeneity of plant development in crops. The use of a proportion of crop lodging does not take the degree of plant-to-plant variability in to account. Empirical evidence from industry indicates that scheduling an optimal lifting time is challenging for crops that develop heterogeneously (Dennis J 2012 pers. com). For these types of crops, a larger proportion of bulbs are likely to be under- or over-mature in production systems based on single pass lifting operations.

Most research on the effects of time of lifting treatments has focused on bulb yield and post-harvest quality. In contrast, very few studies have examined the effect of time of lifting on physiological processes in onions. Nilsson (1980) monitored non-structural carbohydrate concentration and amino-nitrogens of bulbs lifted at regular intervals during bulb growth and during storage. Only minor changes in non-structural carbohydrate concentration were recorded during growth and after storage. However, Nilsson (1980) found increased amino-nitrogen levels in bulbs after lifting, which was attributed to translocation from senescing foliage. The translocation of sprouting inhibitory chemicals from the senescing foliage to expanding bulbs has also been recorded (Stow 1976). Stow (1976) examined the effect of defoliation at time of lifting on the storage life of bulbs and observed that defoliated bulbs sprouted earlier than intact bulbs. Stow (1976) explained this response by the lack of growth inhibitors that would normally be translocated from the foliage to developing bulbs. Previous work had also found that levels of sprouting inhibitors (not identified) fell during storage in line with increases in sprouting (Thomas et al. 1972). The plant hormone, abscisic acid (ABA) has shown to be responsible for dormancy in plant organs such as seeds (Gubler et al. 2005) therefore, Chope (2006) examined the possible role of this plant hormone in sprouting/dormancy response. Chope (2006) applied exogenous ABA analogues to the foliage and bulbs but did not detect an enhanced dormancy response, possibly due to lack of absorption, as tissue ABA content did not increase.

Having originated from the mountainous regions of central Asia (Brewster 2008), the dormant onion bulb is able to withstand unfavourable conditions such hot, dry summers.

Onion bulbing is widely accepted therefore as a stress avoidance response to unfavourable growing conditions recognised using increasing day length and changes in red:far-red light ratios (Tarakanov 2005). Modern commercial crop management is imposed on the onion plants evolved survival strategy. Characteristics associated with early interruption of the plants natural stratagem appear to imbue traits advantageous to counter-seasonal export market requirements, albeit with a yield penalty.

In Tasmania, the production system is designed for the counter seasonal bulb export. This system is characterised by infield curing and long term storage without controlled atmosphere or the use of sprouting inhibitors. As such, maximising storage potential is critical, however, in order for the Tasmanian onion industry to improve scheduling of lifting onion bulbs, there is a need for improved scientific understanding of the effect of time of lifting on subsequent physiological processes. We hypothesize that: 1) the timing of lifting will affect the yield and post-harvest storage of onions and; 2) rapid crop growth leads to high yields but reduced post-harvest storage life.

Materials and methods

All field experiments were conducted in commercial crops grown in the North and North West of Tasmania. Crops were planted on beds with 1.83 m wheel centres (except Crop Pla13 which had 2 m wheel centres) in a ten row configuration with a target stand density ranging between 60 and 80 plants.m^{-2}. Crops were managed using standard agronomic practices based on the advice of an experienced professional agronomist. Briefly, fertiliser

0N-7P-8K was applied at a rate of 1000 kg ha^{-1} and incorporated prior to planting. The crops were side dressed with urea (46% N) at a rate of 190kg ha^{-1} and muriate of potash applied at 100kg ha^{-1}. Insecticides, fungicides and herbicides were applied as required and crops irrigated regularly to field capacity until late bulbing.

Growth rate manipulation

Plant growth rate of two '*Early Creamgold'* onion crops (CGa12 and CGb12 planted on the 4/5/2011 and 8/5/2011, respectively) were manipulated with fertiliser and defoliation treatments (details below). Treatments were arranged in a randomised complete block design (*n=4*) with each experimental unit (plot) consisting of a 5 m length of bed, 1.8m wide. The three treatments were: 1) standard crop management (Control); 2) standard crop management with additional nitrogen (83kg ha^{-1}) applied as urea (46% N) at the 3-leaf, 6-leaf and at initiation of bulbing stages (High N) and; 3) standard crop management with defoliation, which involved the removal of all leaf blades 1 cm above the ground using hedge shears at the 3-leaf stage (Defoliation). Bulb and foliage, fresh and dry mass were recorded at weekly intervals from the commencement of bulbing to a week prior to lifting. Bulb firmness and internal sprout growth were determined post-harvest using the methods described below. All plots were mechanically lifted on the same day (when the crop reached approximately 80% tops-down) and bulbs were cured for 3 weeks in the field.

Time of lifting

The effect of time of lifting on bulb yield and post-harvest storage was examined in five separate field experiments conducted over three growing seasons from 2011 to 2013. Rainfall on a long term average and for the years lifting treatments were applied have been reported on a monthly basis in appendix 1. At each site, the experiment was set up as a randomised complete block design with each experimental unit (plot) consisting of two linear meters of planted bed. Seven treatments were applied by hand lifting based on the date when 80% tops-down would occur as predicted by an experienced agronomist and included: 1) not lifted (UL); 2) lifted at either five weeks prior (5WPre) or; 3) two weeks prior (2WPre) or; 4) one week (1WPre) prior to 80% tops down; 5) lifted at 80% tops down (0WPre); 6) lifted one week post (1WPos) or; 7) two weeks post predicted 80% tops down (2WPos). A summary of the time of lifting experiments for each year and crop is provided in **Table 3-1**. Canopy lodging was monitored at regular intervals for 20 plants per block in 2013, or visually estimated per plot in 2011 and 2012 Plants were considered to have lodged when the pseudostem was no longer self supported in an upright position. The difference between predicted tops-down and actual tops-down percent were noted.

Table 3-1 Summary of time of lifting experiments, conducted in 2011, 2012, 2013 with '*Creamgold'* (CG), Early (E), Regular (R) or the hybrid '*Plutonus'* (Pl). Bulbs were lifted up to five weeks earlier (-5) than the predicted lifting date, and up to two weeks past the predicted lifting date (+2), at which time 80% of the crop canopy was expected to have collapsed. A range of pre- and post-harvest parameters were recorded.

Year	**Blocks (n)**	**Crop ID (date planted)**	**Lifting treatment relative to estimated 80% tops-down in weeks(predicted tops-down)**	**Pre-harvest parameters recorded**	**Post-harvest parameters recorded**
2011	4	CGb11 (R) (18/9/2010)	3WPer, 2WPer ,1WPer ,0 WPer ,1WPos, 2WPos (3/03/2011)	Yield, tops-down %	Respiration rate, (all bulbs cured for 2 weeks post-lifting)
2012	4	CGa12, (E) (4/5/2011) CGb12 (E) (8/5/2011)	3WPer, 0WPer, 2 WPos (29/12/2011)	Yield, tops-down %, fresh and dry foliage and bulb mass	Internal sprout mass and length at 300day post- harvest. All bulbs cured in field until harvesting 2 weeks after the last lifting treatment.
2013	5	CGa13 (R) (13/8/2012) Pla13 (26/9/2012)	2 WPer, 0 WPer, 2WPos (30/1/2013) 5 WPer, 0 WPer, 2WPos (7/03/2013)	Tops-down %, Fresh and dry foliage and bulb mass	Internal sprout mass and length at 150 and 200 days post harvest. All bulbs cured in field until harvesting 2 weeks after the last lifting treatment. (Pla13 +2allowed to cure for 1 additional week).

Growth assessments

Five bulbs were randomly removed from the centre rows (outside of the area used for the yield assessment) of each randomised block at weekly and twice weekly intervals in growth

rate manipulation and time of lifting studies, respectively. Growth was monitored from late bulbing until lifting at 80% tops-down in the growth rate manipulation experiment. In 2012/13, the foliage and bulbs from time of lifting experiments were also sampled on a weekly basis after the treatments had been applied, for up to five weeks. At each sample date, the fresh mass of both bulb and foliage were recorded immediately, and then chopped into segments of less than 2 cm^3. A subsample (100-200g) of this plant material was oven-dried to constant mass at 70°C in an oven to determine dry matter percentage.

Post-harvest assessments

Following field curing, subsamples of bulbs (10-15 kg) were taken and stems trimmed to 2-6 cm, before bulbs were weighed and stored at 19 °C in plastic mesh onion bags. As detailed in **Table 3-1**, bulbs underwent post-harvest storage assessments including internal sprout growth (as described below), respiration rate or bulb firmness using the procedures described below.

Internal sprout growth assessment

Prior to assessment of internal sprout growth, individual bulb weight (g), height (mm) and diameter (mm) were recorded. Height was measured from the base plate to the shoulder of the neck, and diameter at the equator (widest point) using Vernier callipers. The bulbs were then dissected to remove sprout tissue. Sprouts were defined as leaves with a blade to sheath ratio of greater the 0.25 (Brewster 1987). Numbers of sprouts were counted and the length of

the longest sprout was measured using Vernier callipers. All of the sprout tissue was then combined for each bulb and weighed on an analytical balance.

Respiration rate

The respiration rate of bulbs was determined by placing bulbs of a known weight in an 18000 cm^3 sealed plastic container. Air from the bucket was circulated through an infrared red gas analyser (ADC .2250, ADC BioScientific Ltd, Hertfordshire, UK) at a flow rate of 0.6L min^{-1}. The concentration of CO_2 was logged over a 10 minute period and the linear rate of change was used to calculate respiration rate as mmol CO_2 kg^{-1} of bulb $hour^{-1}$.

Bulb firmness

Bulb firmness was assessed using an Instron Materials Testing Platform (5543, Instron, Norwood, MA, USA) with a 100N load cell, based on the method used by Chope (2006). Bulbs were placed on a flat steel plate (13 mm thick) with a locator hole (25 mm diameter) in the centre to stop the bulb from rolling. Bulbs were orientated so that the equator was aligned vertically (perpendicular to the plate). An 8 mm diameter round flat head probe with a cross head speed of 50 mm min^{-1} was used to compress the bulb over a distance of 3 mm. Firmness was then calculated as force per unit distance (N mm^{-1}) based on a linear relationship between force *vs* distance. There was no relationship between bulb width and firmness and thus inclusion of bulb width as a covariate in the analysis did not change the finding (data not presented).

Statistical analysis

Statistical analyses were performed using SPSS (ver. 22). Data were analysed using a general linear model or mixed model, depending on the experiment. Assumptions of normality and homoscedasticity were assessed and data transformed where necessary to meet these assumptions. *Post hoc* tests were computed using Fishers Protected Least Significant Difference (LSD).

A mixed model was used to test the effect of growth modifying treatments with treatment, crop and crop by treatment as fixed factors. Block within crop was used as random effect to test crop effect and the residual was used to test treatment and treatment by crop. Additionally bulb firmness was tested for crop by storage time and treatment by storage duration. Linear regression was used to describe the relationship between lifting time and plot yield.

Results

Growth manipulation

The fresh mass of onion bulbs in the HighN and defoliation treatments was on average 21-23% less than compared with the control treatment for both crops one week prior to lifting (**Figure 3-1 a & b**.). Bulb fresh mass was not different between either nitrogen or defoliation treatments (**Figure 3-1**). At the final sample date, dry matter partitioning to bulbs in the control treatment was greater than in the HighN and defoliation treatments for crop CGa12 but not CGb12 (**Figure 3-1**).

At harvest, which followed 3 to 4 weeks of on-ground curing, the total yield was 18% and 25% lower in the HighN and defoliation treatments, respectively, for crop CGb12 (**Table 3-2**). After storage for 250 days, there were no differences in relative sprout growth among the treatments (**Table 3-2**). However, the relative internal sprout growth was higher for CGa12 than CGb12 (**Table 3-3**).

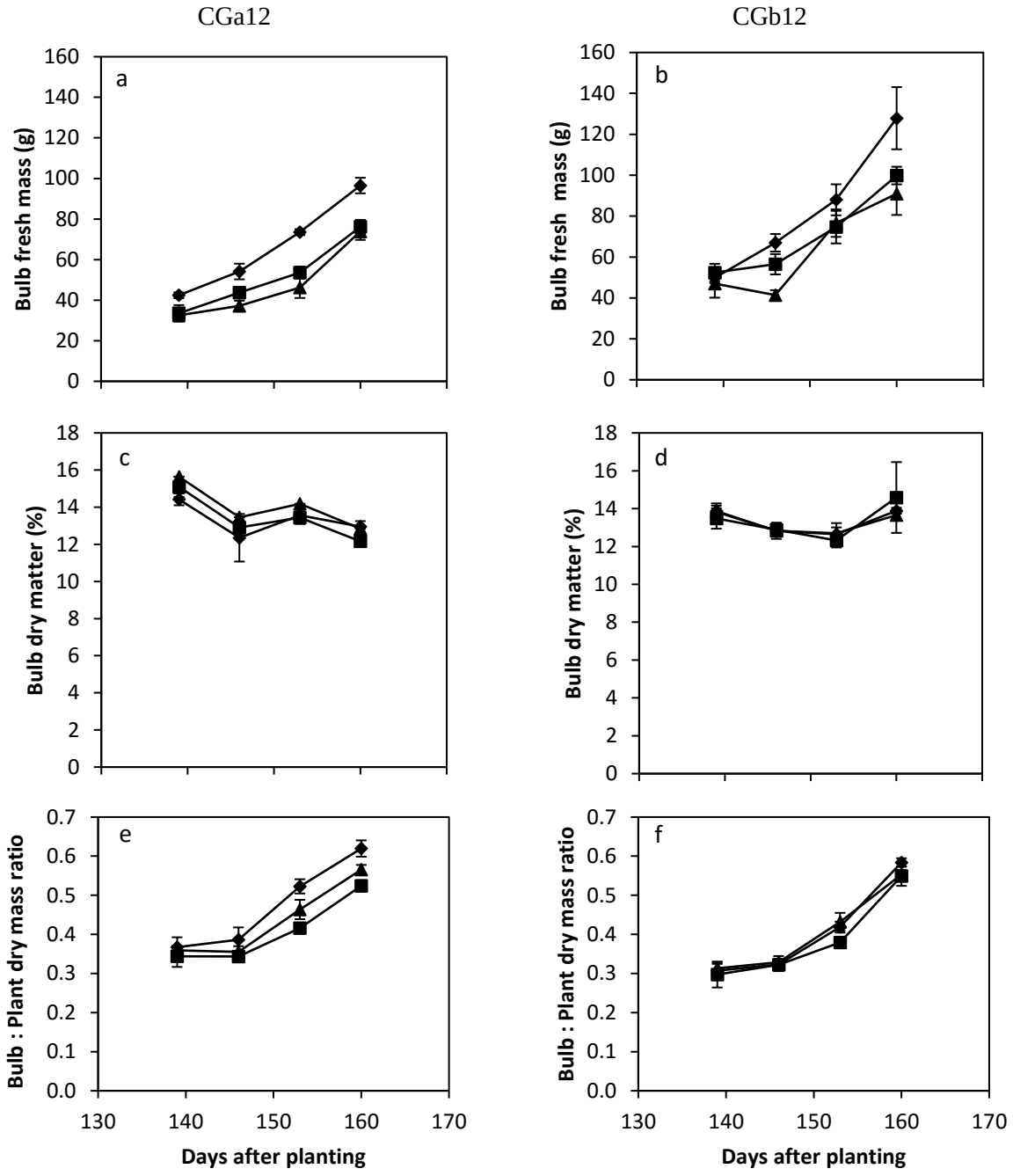

Figure 3-1. The effect of nitrogen and defoliation treatments on onion plant growth. Control treatment (◆), high nitrogen (HighN) (3 x 83 kg/ha Urea) application (■) and early defoliation (Defoliation) at the 3-leaf stage to 1 cm above ground height (▲) on bulb growth of two commercial crops (CGa12 ad CGb12) during the 2011-12 season; mean bulb fresh mass (a & b), bulb dry matter (%) (c & d), Bulb : Plant dry mass ratio (e & f). Error bars indicate ± 1 SEM.

Table 3-2. Effect of crop and growth modification treatments (Nitrogen and Defoliation) on yield at harvest, onion sprout length and mass as a proportion of bulb height and mass after 250 days of storage.

			Yield		Length		Mass	
Fixed Effects	**Num d.f.**	**Den d.f.**	**F Value**	**Sig.**	**F Value**	**Sig.**	**F Value**	**Sig.**
Crop	1	6	21.967	**0.003**	10.311	**0.049**	6.127	0.090
Treatment	2	12	11.934	**0.001**	0.059	0.943	0.200	0.821
Crop*Treatment	2	12	3.602	0.060	0.043	0.958	0.059	0.943

Table 3-3. Effect of nitrogen and defoliation treatments (Cntrol, HighN (three applications of 83 kg/ha Urea) or Defoliation at 3leaf to 1cm above ground height) on total yield (kg m^{-2}) at harvest, and sprout growth 250 days after harvest for two crops (CGa12 ad CGb12). Sprout growth is expressed as mean sprout mass and sprout length as a proportion of bulb mass and height respectively. Mean values are shown ± SEM, n=4.

Crop	**Treatment**	**Yield (kg m^{-2})**	**Sprout mass (%)**	**Sprout length (%)**
CGa12	**Control**	7.8 ± 0.42	1.48 ± 0.14	51.2 ± 3.2
	High N	7.1 ± 0.22	1.45 ± 0.18	51.2 ± 5.6
	Defoliation	7.1 ± 0.45	1.34 ± 0.16	49.1 ± 2.8
CGb12	**Control**	10.2 ± 0.31	1.24 ± 0.13	44.9 ± 3.5
	High N	8.4 ± 0.48	1.27 ± 0.13	43.9 ± 2.6
	Defoliation	7.7 ± 0.14	1.20 ± 0.15	44.2 ± 2.4

Bulbs of crop CGa12 were significantly firmer by 6 ±1 N mm^{-1} than those of crop CGb12 at the start of the storage ($F_{1,6}$ = 41.27; p<0.001) (**Figure 3-2**). There was an interaction between crop and storage duration ($F_{2,36}$ = 26.82; p<0.001); the firmness of bulbs in crop CGa12 decreased during the 100 days after harvest whereas firmness increased in crop CGb12 (**Figure 3-2**). Bulbs from the HighN treatment were softer (27-28N mm^{-1}) than those from the Control and Defoliation treatments at 100 ($F_{2,12}$ = 4.515; p=0.035) and at 250 ($F_{2,12}$ = 6.064; p=0.015) days in storage but there was no interaction between treatments and crop, nor between treatments and storage duration. Bulb firmness did not differ significantly

between the control and Defoliation treatments ranging from 29 to 31 N mm^{-1} in firmness at the final sample date for both crops.

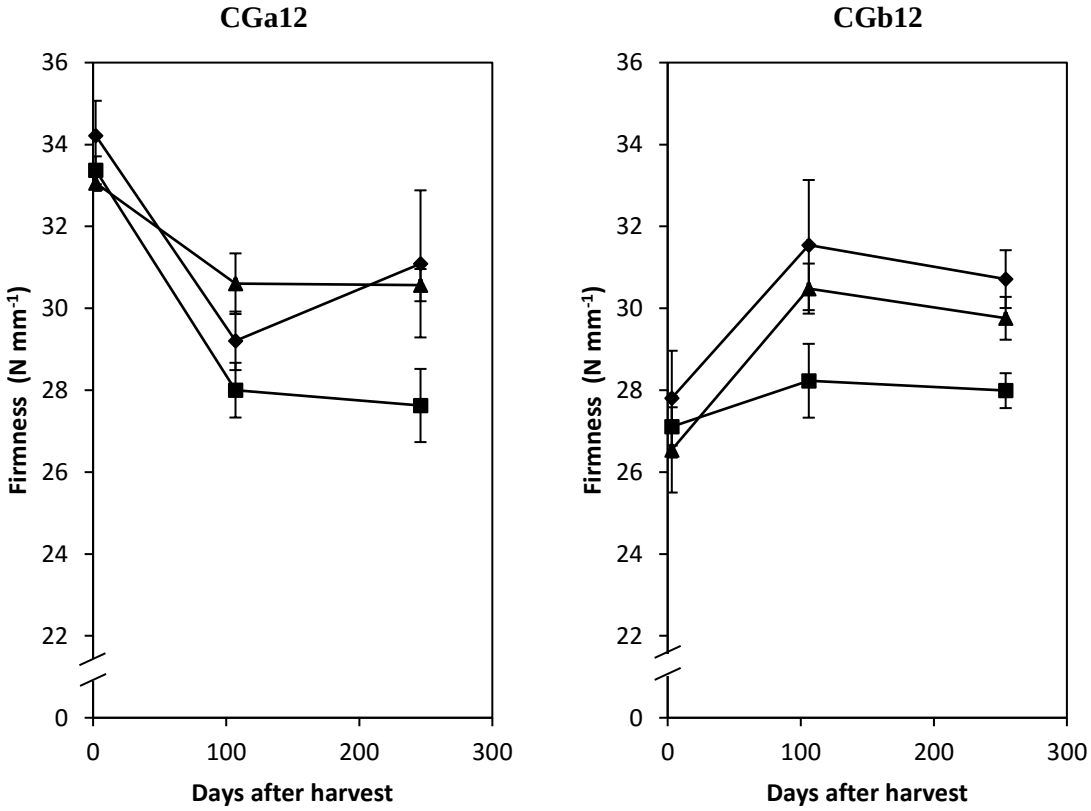

Figure 3-2. Effect of nitrogen and defoliation treatments on bulb firmness during storage for crops CGa12 (a) and crop CGb12 (b). Treatments applied were Control (◆), HighN (three applications of 83 kg/ha Urea)(■) and Defoliation (▲) at 3leaf to 1cm above ground height. Error bars indicate ± SEM, n=4.

Time of lifting

Bulb growth and yield

The fresh mass and dry matter % of both crops increased after lifting in the early lifting treatments 5WPre and 2WPre (**Figure 3-3**). Specifically, the fresh mass of '*Plutonus*' bulbs increased by 15% (14g) in the 5WPre treatment (**Figure 3-3.b**). In contrast, the fresh weight of '*Regular Creamgold'* bulbs increased by almost 30% (24g) in the 2WPre treatment in crop CGa13 (**Figure 3-3**.a). There was also a >20% increase in bulb dry matter % after early lifting treatments 5WPre and 2Wpre in both crops (**Figure 3-3**.b&c) and a 35% increase in the bulb proportion of total plant dry matter in crop Pla13 in the 5WPre treatment (**Figure 3-3**.f). Changes on this scale were not obvious for crop CGa13 in the 2WPre treatment (**Figure 3-3**.e)

There was a positive relationship between time of lifting and total yield, with yield (± 1 SE) increases ranging from 0.39 (± 0.087) to 0.76 (± 0.110) kg m^{-2} for every week of delayed lifting with the magnitude varying for crop (**Figure 3-4**).

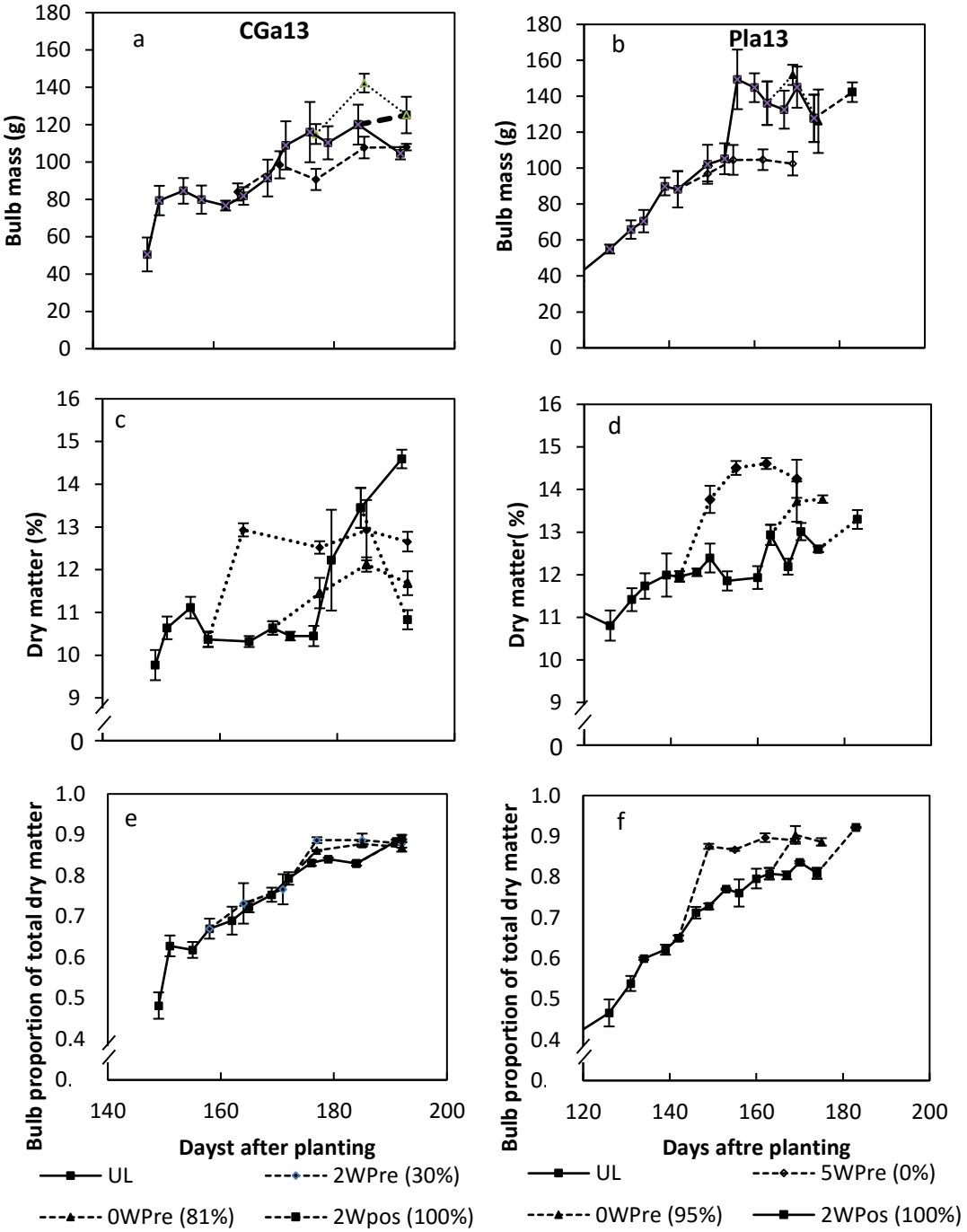

Figure 3-3. The effect of time of lifting treatments on bulb parameters in the 2012-13 season for crops CGa13 and Pla13 including bulb fresh weight in crops (a & b); bulb dry matter (%) (c & d); and bulb dry weight as a proportion of total plant dry weight (e & f). Bulbs were either not lifted (UL) or lifted relative to predicted 80% tops-down at five weeks prior (5WPre), two weeks prior (2WPre), at predicted lifting (0WPre) or two weeks post predicted (2WPos). Dashed line indicates that bulbs have been lifted. Error bars indicate 1± SEM, parentheses in the legend indicate actual tops-down (%) at lifting.

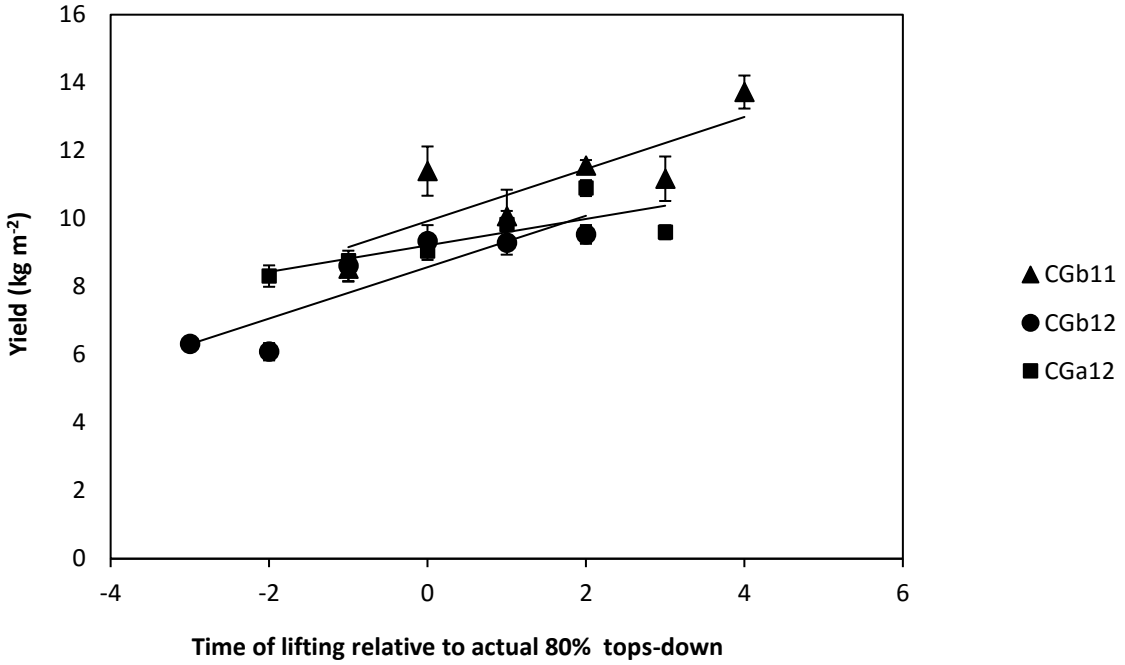

Figure 3-4. The effect of time of lifting experiments on mean yield (kg m^{-2}) for three '*Creamgold*' crops (CGb11, Cgb12, CGa12) lifted two (-2) weeks prior to the date at which 80% of the crop canopy collapsed, to four (+4) weeks after this time. Error bars indicate ±1 SEM. Linear regressions: CGa12 y = 0.39X + 9.21, $p<0.001$, $R^2 = 0.63$; CGb11 y = 0.76X + 9.93, $p<0.001$, $R^2 = 0.69$; CGb12 y = 0.76X + 8.57, $p<0.001$, $R^2 = 0.80$.

Post-harvest bulb quality

Internal sprout length and mass as a proportion of bulb height and mass of stored bulbs was lowest in bulbs that were lifted just prior to 100% tops-down (95 – 100%), compared with earlier or later lifting treatments (**Figure 3-5**). This trend was observed across the two seasons (2012 and 2013) and for the hybrid '*Plutonus*' and open-pollinated '*Regular Creamgold*' cultivars, and with varying storage times (150 to 300 days) post lifting. Bulbs from crop CGa13 did not display the same internal sprout growth response pattern at 200

days after harvest. Crop CGa13 was the only crop lifted at *ca.* 80% canopy collapse at the predicted lifting date (Table 3-3).

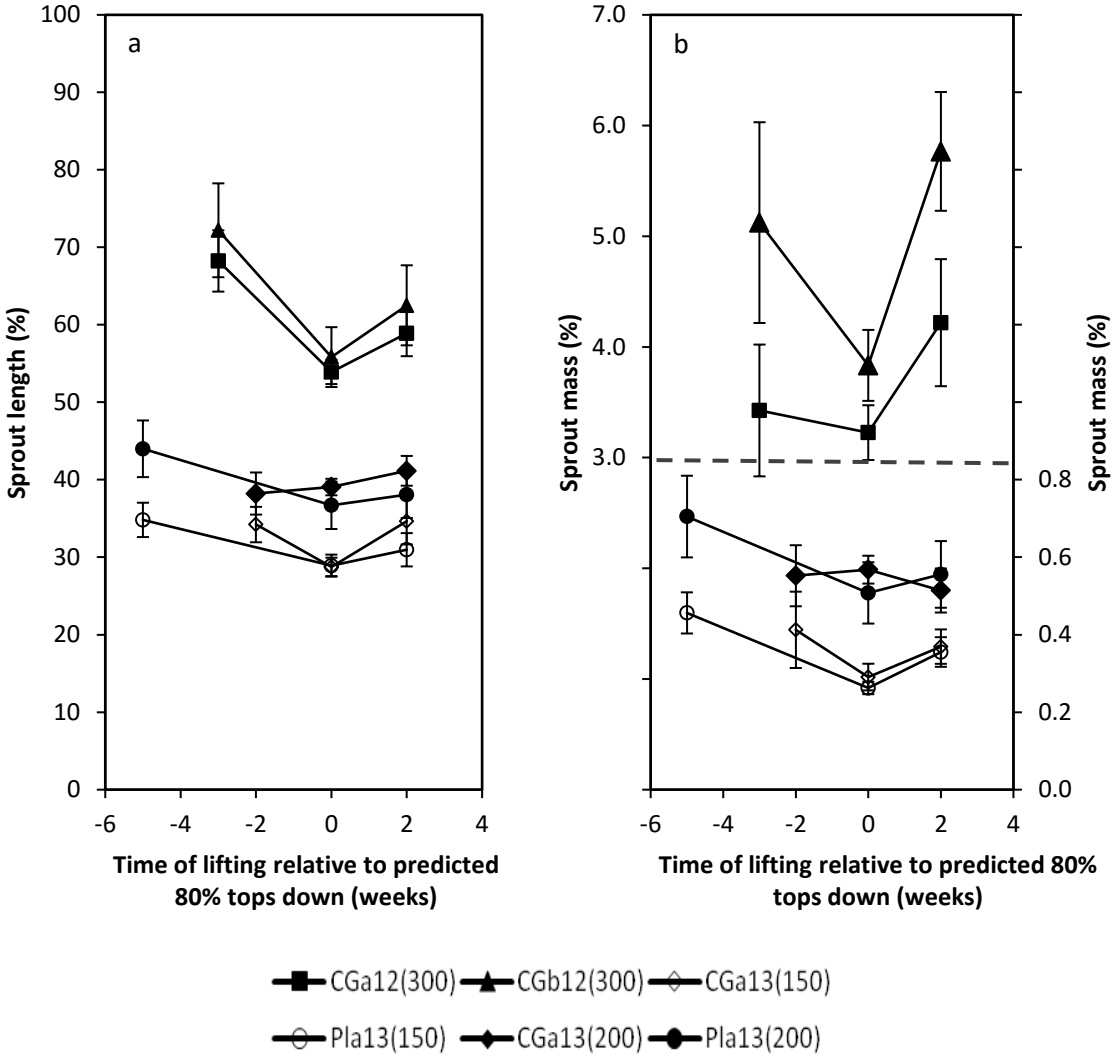

Figure 3-5. Effect of time of lifting experiments on bulb sprout parameters for '*Creamgold*' and '*Plutonus*' crops from the 2011/12 and 2012/13 seasons. (a) Sprout length as a percentage of bulb height (b) sprout mass as a percentage of total bulb mass. Parentheses indicate the number of days after harvest. Error bars indicate ±1 SEM. Dashed line indicates the transition between Y axis scales.

Table 3-3. Actual tops-down percentage when treatments were applied relative to the predicted 80% tops-down in crops CGb11, CGa12, CGb12, CGa13 and Pla13.

	Weeks relative to predicted 80% tops-down						
Crops	-5	-3	-2	-1	0	1	2
CGb11		0%	81%	90%	98%	100%	100%
CGa12		0%			100%		100%
CGb12		4%			95%		100%
CGa13			30%		81%		100%
Pla13	0%				95%		100%

Similar onion respiration rates were recorded across all lifting times for '*Regular Creamgold*' bulbs at 68 days after harvest (**Figure 3-6**). After 267 days in storage, bulbs harvested at 90% tops-down had the lowest respiration rate (0.05 m mol kg^{-1} hr^{-1}). Bulbs at this stage had respiration rates that were approximately half the rate of those lifted 1 week pre (0.1 m mol kg^{-1} hr^{-1}) and 4 weeks post (0.12 m mol kg^{-1} hr^{-1}) actual 80% tops-down (**Figure 3-6**). This difference in respiration rate is consistent with that observed with sprout growth (**Figure 3-5**).

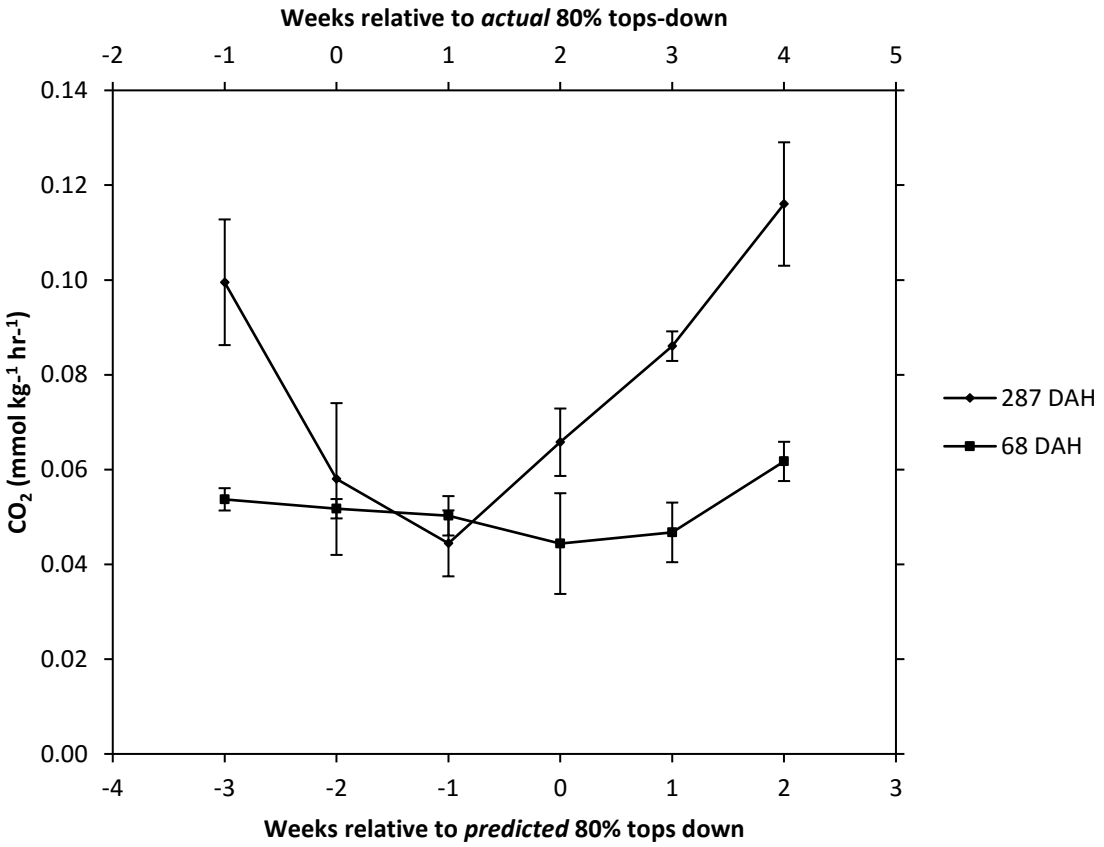

Figure 3-6. Respiration rate of '*Regular Creamgold*' bulbs at 68 and 287 after days after harvest. Bulbs were lifted relative (weeks) to the predicted 80% canopy collapse treatment. The upper X axis shows weeks relative to actual tops-down (%). Error bars indicate ±1 SEM, n=4.

Discussion

In this study, the total yield of field cured onions increased as lifting was delayed but storage potential was greatest when lifted at 80-100% tops-down, highlighting the importance of lifting onion crops at an optimal stage of development. Usually, lifting either side of this window resulted in greater internal sprout growth, loss of marketable yield both at harvest and in storage, and higher respiration rates during long term storage. The results also indicated that lifting should occur at 80% tops-down at the earliest as lifting prior to 80% tops-down is likely to be more detrimental to the yield-quality nexus, especially for bulbs intended for long term storage. Furthermore, there may be significant advantages in yield and storage attributes if lifting is conducted at 90% tops-down or higher, as similarly postulated by Wright (1993). As expected, the defoliation treatment reduced the rate of growth and final yields; of some note was that the HighN treatment also decreased yield to the same extent as foliage removal, while also resulting in softer bulbs.

Collapse of the onion pseudostem and plant canopy is a clear indication that the onion has reached a milestone in its life-strategy. The collapsed pseudostem and foliage is no longer optimal for harvesting light (Brewster et al. 1986), and the relatively fast onset of leaf senescence would seem to suggest that metabolic activity is aimed at increasing the bulbs storage capacity to endure a resting phase (Gracie & Boersma 2009). This is also attested to by the plants response to disruption through early lifting; in this situation of enforced rest, a similar response occurs, where in reaction to upheaval, the onions were able to rapidly redistribute dry matter from leaf to bulb, increasing bulb mass by almost 30%. This was

clearly seen in crop Pla13, where after lifting five weeks prior to the predicted lifting date, the bulb proportion of total plant dry mass increased sharply, indicating leaf material was reassimilated into the bulb. In contrast, dry matter allocation to bulb was unaffected in the later lifting treatments suggesting the majority of bulbs were transitioning into a dormancy phase. This transition is also attested to by the least sprout growth when lifting after 90% tops-down, and the heightened sensitivity to earlier lifting again suggests that sprout development may also reach an optimal stage associated with pseudo stem collapse.

The premise of the growth manipulation treatments was that altering growth rates, and the subsequent changes in canopy structure, weight, and neck thickness may influence crop canopy collapse. The Defoliation and HighN treatments resulted in slower growth, reduced bulb yield and increased partitioning of photosynthates to the foliage, as evidenced by the observed increased dry matter allocation to foliage. Nitrogen application has been shown to increase biomass allocation to foliage growth and affect light quality, reducing the red:far red light ratio which leads to earlier bulbing (Brewster 1990). As foliage dry weight was greater under the HighN treatments in Crop CGb12, indicating a heavier canopy, it is plausible that the reduced yield was an indirect consequence of earlier bulbing. This is unlikely to have been the case where the foliage was removed during early growth in the Defoliation treatment, where the red:far-red ratio would have been increased.

The length or mass of sprout tissue during storage was unaffected by either HighN and or Defoliation treatments. There were differences between crops in the initial bulb firmness

however this was not due to treatment effect. After 100+ days in storage, the difference in bulb firmness between crops were reduced and the differences between treatments increased with the control treatments producing the firmest bulbs in both crops. This indicates that bulb firmness can differ between crops, change during storage and, can be affected by crop management though these differences may not be apparent in the firmness test used soon after harvest.

Sprout growth is a fundamental component of the developmental transition of onion bulbs from dormancy to reproductive growth. The sprout length percentage and the sprout mass percentage were smallest in the 0WPre treatment at 300 days in 2012 and at 150 days in 2013. This could mean that the bulbs remained dormant for longer and/or the sprout growth within the bulbs had sprouts that grew at a slower rate. The differences between 150 and 200 day sprout assessments indicates that sprouts grew during this period. As the differences' in sprout length and sprout mass percentage did not persist through to 200 days the sprouts appear to have grown at different rates with the bulbs with the predicted 80% lifting treatment having faster sprout growth than those with early lifting treatments. This is counter to findings by Pak et al. (1995) who reported a lack of sprout growth dormancy and a linear sprout growth in a single crop and lifting time.

The lifting treatments had little impact on the respiration rate 68 days after predicted 80% tops-down however differences in the respiration rate became apparent later during storage at 287 DAH. In particular, the respiration rate was approximately double to that of the control in

the 3WPre and 2WPost treatments. This result suggests that the metabolic activity of bulbs in prolonged period of storage increases if they have been lifted earlier or later than the optimum lifting time. Some of the differences in respiration rate between the different times of harvest may be attributable to disease. The latest harvested treatment had the greatest number of bulbs removed due to infection or externally visible sprout growth. Wright (1993) showed that later harvested bulbs were more susceptible to rot. Climate may also have been a factor with late lifted bulbs exposed to more wet weather prior to harvest. This highlights the challenge of experimental design in testing the timing of lifting in a variable climate.

Despite the importance that stage of physiological development at lifting plays in determining yield and post-harvest storage potential in cool temperate climates, accurately determining the stage of physiological development remains a key challenge of optimising the scheduling of crop lifting. The findings from this study clearly show that the percentage of crop canopy has lodged can be linked to bulb physiology and storage life, yet there are still some concerns with the accuracy of using tops-down% as a visual marker of physiological development. In particular, pseudostem lodging can be influenced by wind and there is difficulty in predicting the pseudostem lodging rate of crops. Given the level of crop development variability, field staff my need to monitor crops more frequently to differentiate between 80 and 90% tops down. Furthermore, canopies which collapse have been observed by experienced commercial field staff to return upright if knocked down before they are sufficiently developed (Smallbon pers. comm.). Therefore, there is a strong case to investigate in more detail the physiological processes linked to canopy collapse, to develop an alternative method for monitoring onion

crop development for the optimisation of the timing of lifting. This is the objective of chapter 4.

Chapter 4: Developmental control of pseudostem collapse in bulbing onions (*Allium cepa* L.)

Abstract

In temperate climates, commercial onion crops are typically lifted when the foliage of 80% of the crop has collapsed, referred to as 'tops-down'. Although tops-down is used as a developmental marker of a crop's readiness for lifting, there is little understanding of the physiological and biomechanical functions that govern pseudostem collapse and how this relates to yield and bulb quality. Measures of dry matter partitioning, tops-down, pseudostem deformation under transverse load, and estimated self-weighted moment were used to build a mechanistic understanding of pseudostem collapse. The estimated self-weighted moment of the canopy decreased slightly prior to pseudostem buckling and therefore did not explain canopy collapse. Instead, softening of pseudostem tissue at the neck, as evidence by increased sensitivity to deformation, explained the timing of canopy collapse. Logistic regression analysis demonstrated that the probability of pseudostem collapse was strongly related to the softening of the base of the pseudostem as determined by measuring deformation under transverse load. Pseudostem deformation under transverse load could therefore be used to schedule crop lifting in commercial production systems and to estimate variability within and among onion crops.

Introduction

Pseudostem lodging is a part of the first year of the onion life cycle (Rey et al. 1974), and interrupting onion plant growth and development by lifting has been shown to affect bulb yield, quality and storage potential (Chapter 3 ; Sargent et al. 2001; Suojala 2001; Wright et al. 2001). Mondal et al. (1986a) defined maturity date as, "*the date at which 80% of plants have flaccid pseudostem that cannot maintain the leaf blades erect*". Using this concept the studies cited above used the collapse of the pseudostems (tops-down) as a marker for development and identified 50-80% to be the optimal stage to lift onion crops in temperate regions, and these recommendations have been adopted widely among commercial operations and researchers. However tops-down can be influenced by external factors such as wind (Heath 1945), potentially biasing assessments of crop development, and when considered on a individual plant basis is a binary variable, limiting capacity to extrapolate one plant's development to forecast the duration of the event across an entire crop. Despite this, "tops-down" remains a commonly utilised indicator of crop development in both commercial bulb production and crop research.

Although an important marker for physiological development, only qualitative descriptions of the process of crop lodging in onions have been published. For example, based on work originally conducted by Rey et al. (1974), Brewster (2008) described this developmental stage as *'fall-down' or 'soft neck'. "The neck or pseudostem becomes hollow as new leaf blades cease to grow within it, and the neck tissue loses turgidity and soften so that the foliage collapses under its own weight*." This description alludes to an increased likelihood of

mechanical failure via. Brazier buckling that can affect hollow cylindrical structures. An improved understanding of the mechanisms which govern pseudostem collapse may enable improved scheduling of crop lifting based on crop development in order to maximise storage potential and yield without needing to rely on percentage tops-down (Chapter 3).

Lodging in plants occurs either through a failure of root anchorage (root lodging), or stem failure (stem lodging) (Crook et al. 1994). Lodging due to anchorage failure occurs when the moment transferred through the stem exceeds the strength of root anchorage. Lodging caused by both mechanisms have been modelled for various crop species including barley (Berry et al. 2006), wheat (Crook & Ennos 1994) and trees (Niklas 2000) but not for onion plants. These studies highlighted that the centre of gravity and the above ground mass are key factors in lodging of plant stems. For example, Crook and Ennos (1994) showed that the centre of gravity increased during wheat development peaked during grain fill. Where both the mass of the foliage and centre of gravity are known, an estimate of the self-weighted moment can be calculated (ESWM). As ESWM increases the load imposed on the stem increases and will at some point exceed the stem's (or pseudostem's) critical load, causing the plant to lodge.

Tops-down in onion bulbs is not associated with root lodging as bulbs remain upright and rooted in the ground (Rey et al. 1974). Instead, observations indicate 'tops-down' is a result of pseudostem buckling and is consistent with the stem lodging noted for crops where anchorage exceeds the strength of the stem (Crook & Ennos 1994). Stem lodging can occur as Euler buckling (long wave) which causes a slender vertical column to deform into a curve

under an applied force or Brazier (short wave) buckling, evident as a localised crimp structural failure, and which can occur following Euler buckling (Niklas et al. 2012). These types of stem buckling are more common in plants with hollow stems. Although hollow stems have a relative strength to weight advantage over solid stems of the same material, which may have ecological and evolutionary advantage (Spatz et al. 2013), they are more prone to Brazier buckling. The susceptibility to this buckling because the anisotropic matrix of plant tissue in herbaceous stems provides resistance to compressive stress (Schulgasser et al. 1997). Models of buckling failure of plants with hollow stems have been developed but not for plants with more complex anisotropic plant structures such as the multiple layers of an onion pseudostem (Spatz & Niklas 2013; Spatz et al. 1990).

The onion pseudostem is a complex tapered cylinder, formed by multiple layers of tubular sheath tissue. As the number of sheaths comprising the pseudostem decreases acropetaly, the diameter of the pseudostem decreases. The inner most layers also contain the leaf blades of emerging leaves (Brewster 2008). Each leaf blade is orientated approximately 180° to the previous emerged leaf blade, elongating the pseudo-stem along this axis, and creating a degree of ovalisation in the transverse plane. This ovalised cylinder of tissue is akin to a tapered vertical beam, firmly attached to a bulb at its base, with cantilevered leaves that progressively have less mass as the pseudostem diameter decreases. Although the pseudostem may already be partially ovalised due to the distichous phyllotaxis, further ovalisation beyond the plastic limit may cause Brazier buckling. Here, further ovalisation may result from structural deformation in the transverse plane in response to a bending moment, when the

walls perpendicular to the direction of the applied force move toward the neutral plane (Spatz et al. 199). Applying these concepts of Brazier Buckling to Rey et al. (1974)'s description of 'soft neck,' we postulate that an softening of tissue of the pseudostem, precipitates the occurrence of pseudostem collapse by reducing the resistance to transverse ovalisation under load.

The aim of this research was to elucidate the mechanisms underpinning pseudostem lodging during the final stages of bulb expansion. Specifically, the study sought to determine whether pseudostem collapse is due to stem softening and/or increases in self-weighted moment. An improved understanding of these processes will assist prediction of canopy collapse in relation to crop development, and possibly reliable tools for the scheduling of crop lifting.

Materials and methods

Field sites

Two commercial onion crops, one '*Regular Creamgold'*, hereafter referred to as crop CGa13 and '*Plutonus*' (a *Pukekohe Longkeeper* hybrid) cultivar, hereafter referred to as crop Plb13 were direct seeded into beds (1.84 and 2.00 m wheel centres respectively) on the 13 August and 26 September 2012, respectively, in the North West of Tasmania. The beds had a eight row configuration that aimed to achieve a target stand population of 70 to 80 plants m^{-2}.

20 plants from each of five adjacent beds (each 5 linear metres, referred as blocks, n=5,100 plants in total) were marked prior to the commencement of lodging. Crop lodging was monitored by counting the number of plants with collapsed psuedostems for each block 1-2 times per week from 2-6 weeks prior to the commencement of lodging through to 100% tops-down. In addition, six plants were randomly sampled from each of the 5 beds at 3 to 7 day intervals just prior to dawn, and plant properties relevant to pseudostem lodging were recorded as described below. Additional assessments of fresh and dry mass were carried out in the week after 100% tops-down.

Growth partitioning and estimated self weighted moment

A total of 25 plants at each sampling date were used to determine dry mater partitioning and ESWM. Each bulb was cleaned of soil and roots removed. Foliage was separated from bulbs by severing at the point where the pseudostem begins widening to form the bulb. The fresh

mass of bulb and foliage fresh mass were recorded. Sub-samples of fresh foliage and bulb tissue (100-200 g) was weighed before drying to a constant dry weight in a drying oven set at 70°C, before reweighing to determine dry matter percentage and moisture content.

The chlorophyll content of the youngest emerged leaves of 3 plants from each of the 5 blocks was measured three times in each plant using a SPAD chlorophyll content meter (SPAD-502 Konica Minolta, Osaka, Japan). Due to the tubular structure of onion leaves, a vertical incision was made along the length of the leaf blades to enable readings through a single layer of leaf tissue.

The location of the foliage centre of gravity was determined using the method employed by Crook and Ennos (1994). The point at which the foliage balanced horizontally on a thin straight edge was found and the distance back to the base of the pseudostem recorded.

Estimated self-weighted moment (ESWM) at 30° was calculated using the formula:

$$\mathrm{ESWM} = \sin\theta hmg$$

Where θ is the angle relative to vertical, *h* is the height of the centre of gravity, *m* is the mass of the lodging stem and *g* is the acceleration due to gravity (Crook & Ennos 1994).

Mechanical properties of pseudostems

The degree of ovalisation under transverse load was recorded from one of the randomly sampled plants per block. Ovalisation of a circle under transverse load can be quantified by dividing *a*: the radius of the pseudostem at the narrowest point and *b*: the radius perpendicular to the narrowest point (**Figure 4-1**).

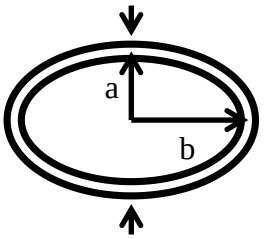

Figure 4-1. From (Spatz et al. 1990)

A 15 mm long segment of the base of the pseudostem from each plant, immediately above the inward tapering from the bulb was carefully excised using a razor blade. These sections were orientated vertically in a custom built test stand between two 11mm thick polyethylene plates. A force gauge (Aliyiq 20 Newton, China) was attached to one plate while the other was attached to a rod actuated by a leaver. When the lever was pushed, a transverse force was applied to the pseudostem section and measured by the force gauge on the opposing side (**Figure 4-2**). A digital camera (Cannon PowerShot A530) was mounted above the test stand and graph paper comprising of 1 mm grid was attached in the same plane for use in scaling elements of the image. Images were then taken at transverse loads of 0, 4, 8, 12 and 16 N.

The diameter of the stem was then measured along the vector component of the applied force (a) and perpendicular (b) to it. The ratio of diameter measurements was then calculated to indicate how circular the pseudostem was at the applied load using the formula;

$$Deformation\% = \left(1 - \frac{a}{b}\right) \times 100$$

Deformation of zero implied that there was no deformation and a ratio of 0.5 implied that the segment is twice as wide as it is thick. The lodging status (upright or lodged) of each sampled plant used to determine deformation was also recorded.

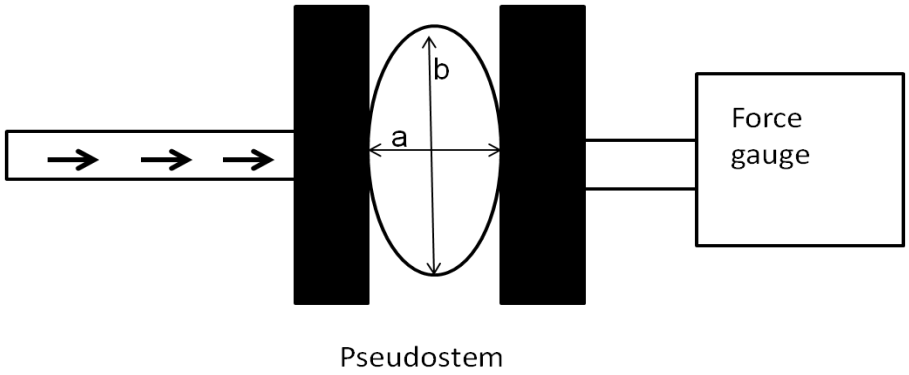

Figure 4-2. Diagrammatic representation of the pseudostem segment testing platform. Force was applied from one side of the pseudostem segment and measured on the opposing side.

Statistical analyses

Statistical analyses were performed using SPSS (ver. 22). Data were summarised using descriptive statistics and a binary logistic regression was used to create a model for predicting

the likelihood of a plant pseudostem lodging based on the degree of softening (as measured by deformation under 4 N transverse load). Other variables for predicting pseudostem lodging using binary logistic regression were assessed and were found non-significant ($P<0.05$) and therefore not reported.

Results

Tops-down and growth partitioning

Crop lodging occurred over 31 days (138 to 169 DAP) for crop Pla13 and 27 days (149 to 176 DAP) for crop CGa13 (**Figure 4-3**a). In both crops, mean foliage fresh mass declined by approximately 70% over the lodging period (**Figure 4-3**b), and this was accompanied by a reduction of foliar dry matter (**Figure 4-3**c) and moisture content (**Figure 4-3**d). Conversely, mean bulb fresh weight increased by approximately 41% across both crops (**Figure 4-3**b). Most of the decline in foliage moisture content occurred after 80% tops-down at 156 DAP, and halved to less than 45% by one week post 100% tops-down (**Figure 4.3**d). Bulb moisture content remained relatively constant, reducing by only 1-5% by the end of the measurement period (**Figure 4-3**d). During the same period, the dry mass of the bulbs approximately tripled from 5 to 15 grams (**Figure 4-3**c).

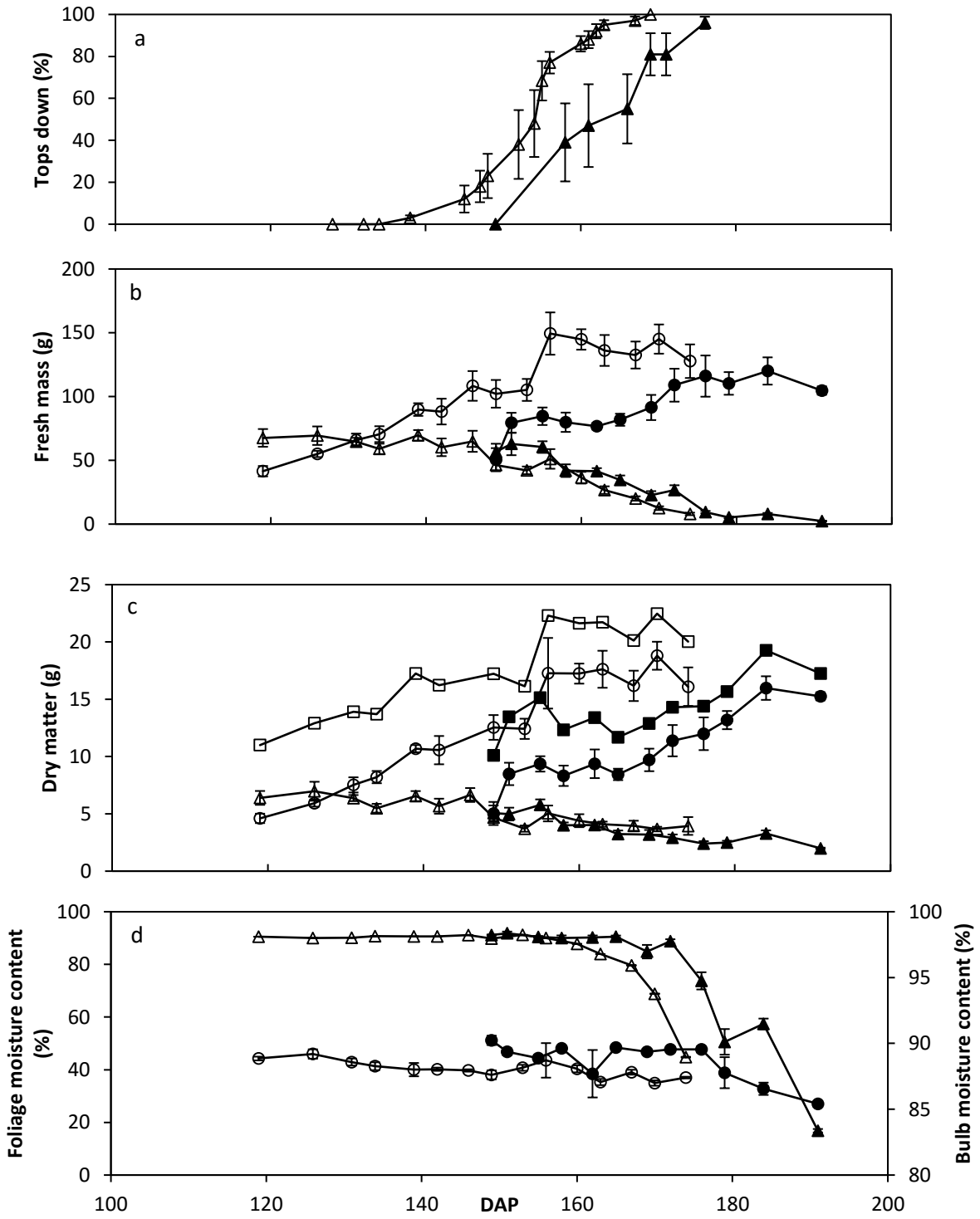

Figure 4-3. The mean (a) tops-down %, (b) fresh foliage and bulb mass (g), (c) dry foliage and bulb mass(g), and (d) percentage moisture content of foliage and bulb tissue for crops CGa13 (closed symbols) and Pla13 (open symbols). Foliage (triangles), bulbs (circles) and total plant (square). Error bars indicate ± 1 SEM, n=5.

The location of the foliage centre of gravity for crop Pla13 remained relatively constant at 17-18 cm above the bulb from 119 DAP until 156 DAP (77% tops-down) after which it declined to 11.6 cm (**Figure 4-4**b). This reduction in the centre of gravity combined with the reduction in foliage fresh mass over the period resulted in a rapid decline in the ESWM from > 0.06 Nm at < 20% tops-down to < 0.03 Nm at > 80% tops-down in both crops (**Figure 4-4**c). The partitioning of dry matter between the bulb and foliage was approximately equal at the start of the observation period for both crops when none of the crop had lodged, with 1 gram of dry matter in the bulb for each 1 gram of foliage dry matter (**Figure 4-4**d). This ratio increased as the crop matured to a ratio of greater than 5:1. This pattern of dry matter partitioning was similar in crop CGa13 relative to the percentage of crop pseudostem lodging.

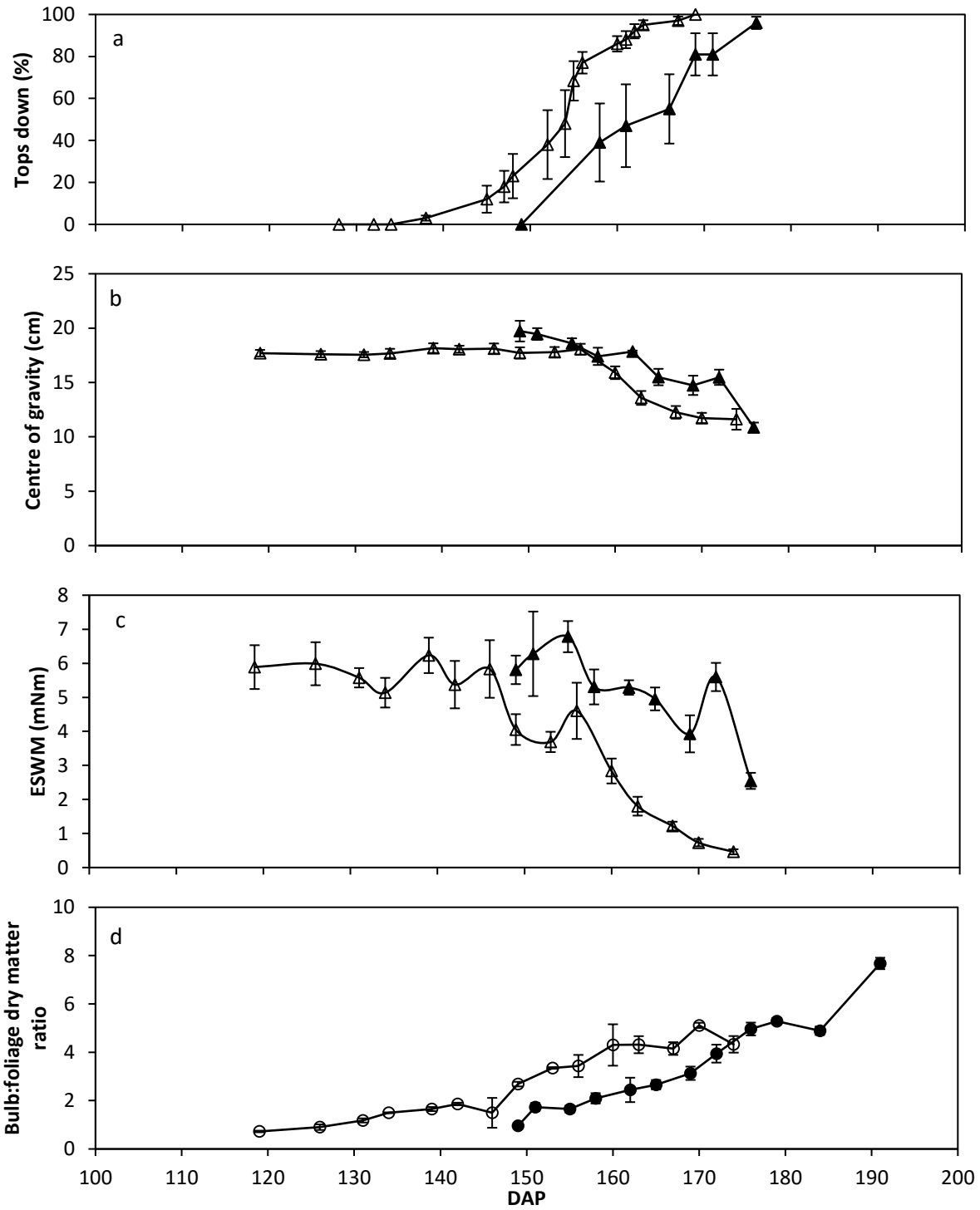

Figure 4-4. Means of; (a) percentage of plants with lodged psudostems, (b) centre of gravity, (c) estimated self-weighted moment, (d) bulb:foliage dry matter ratio for crops CGa13 (closed symbols) and Pla13 (open symbols), foliage (triangles), bulbs (circles) and total plant (square). Error bars indicate ± 1 SEM, n=5.

The chlorophyll content did not begin to decline until 149 day after planting in crop Pla13 when approximately 23% of the crop had lodged and reduced from 31.5 to a minimum of 21.7 SPAD at 100% tops-down. The chlorophyll content in crop CGa13 was not determined prior to lodging, however it decreased from 25.4 SPAD at 55% tops-down to 17.1 at 100% tops-down (**Figure 4-5**.b)

The fresh weight of the 15 mm long fresh neck segments from the base of the pseudostem used to determine mechanical properties decreased from a mean maximum of 2.8-2.9 g prior to lodging to less than 0.5 g in both crops once the crops had passed 90% tops-down. There was an increase in the deformation of the pseudostem segments under 4 newtons of transverse load, with deformation increasing from 123 DAP (before the beginning of tops-down) and doubling from 0.3 to 0.6 over the two week period from 149 DAP in both of the crops (**Figure 4-5**.d). This occurred at the same time as the increase in tops -down percentage from 23% to greater than 85%.

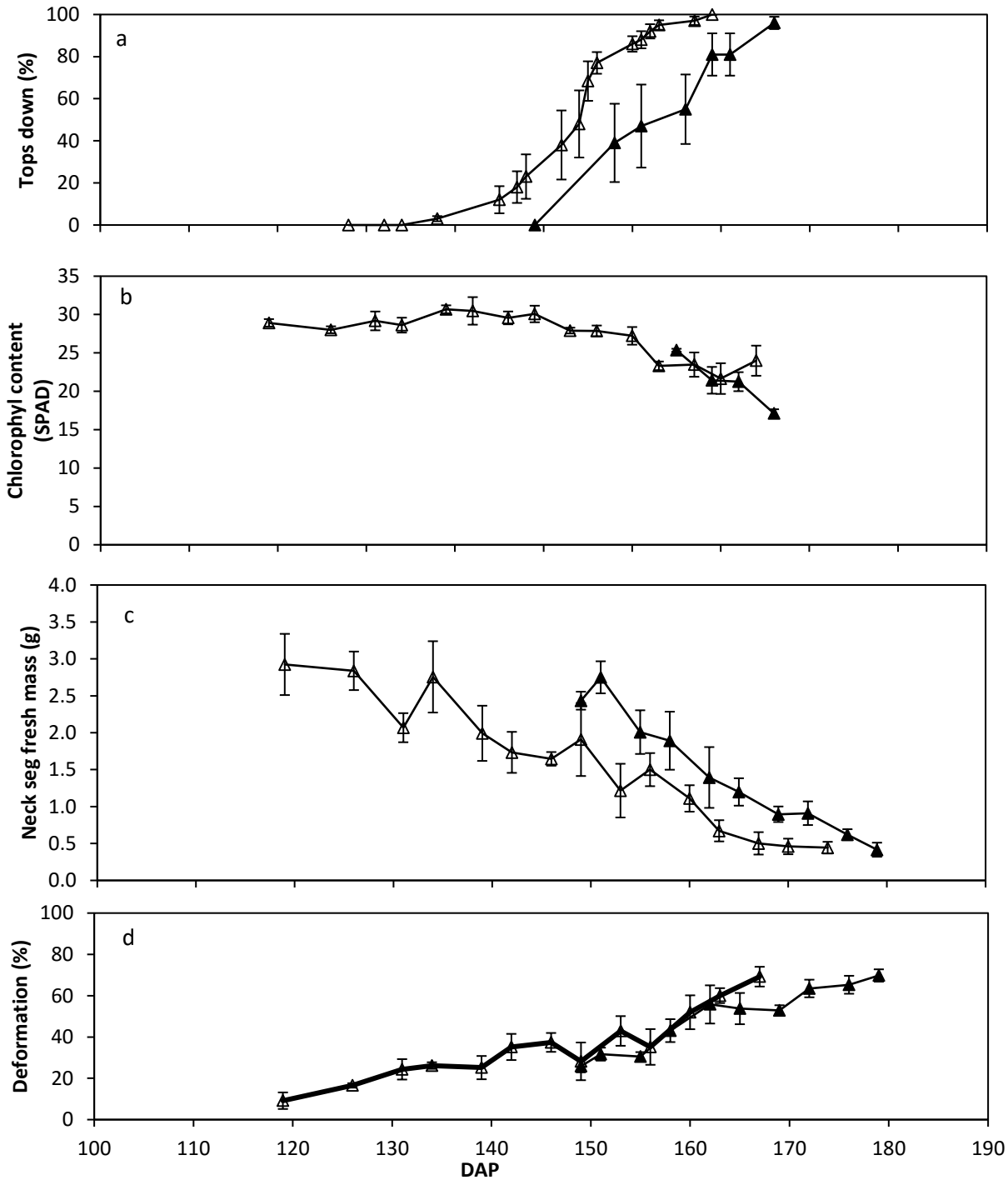

Figure 4-5 Means of; (a) Percentage of plants with lodged pseudostems, (b) Chlorophyll content (SPAD), (c) fresh mass of 15 mm pseudostem neck segments, and (d) Deformation % of pseudostem under 4N transverse load for crops CGa13 (closed symbols) and Pla13 (open symbols), foliage (triangles), bulbs (circles) and total plant (square). Error bars indicate ± SEM, n=5

The relationship between pseudostem softening and lodging

Binary logistic regression was used to evaluate the predictive value of deformation % at 4 Newtons transverse load on the probability of pseudostem lodging. The model; Logit (p) $= 0.231 \times$ Deformation % $- 10.577$ (χ^2 =111.84 (N=113); P<0.001; 85% Nagelkerke R^2) correctly classified 93.8% of cases. The probability of a pseudostem lodging was 50% when the deformation was equal to 46% (**Figure 4-6**).

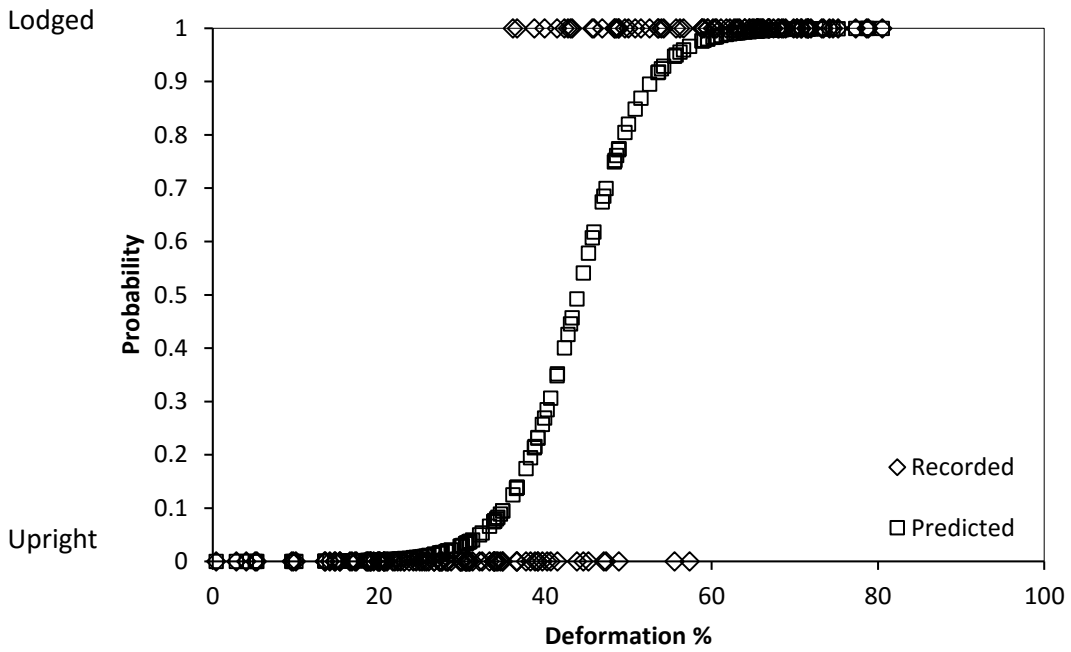

Figure 4-6. The predicted probability of lodging using the parameter deformation at 4N transverse load and the recorded lodging status.

Discussion

In this study, tissue softening at the base of the pseudostem was found to be the primary antecedent of pseudostem collapse. The softening of the neck tissue was evident from the observed increase in deformation (ovalisation of the pseudostem) and decrease in fresh weight of neck segments prior to the beginning of lodging. In contrast, the key components of ESWM, foliage mass (fresh weight) and foliage centre of gravity, remained relatively stable throughout the early measurement period and decreased after 70% tops-down. This finding contrasts with other crops such as wheat and rice, where the probability of lodging increases as ESWM increases during grain fill (Crook & Ennos 1994; Moldenhauer et al. 1994).

Several pieces of evidence are provided to support the hypothesis that the lowering of resistance to ovalisation is closely related to canopy collapse. Firstly, the lowering of resistance coincided with canopy collapse. This is consistent with Rey et al. (1974) who concluded that loss of turgidity and an increase in internal air space of the pseudostem were key developmental changes occurring ahead of canopy collapse. Secondly, the physical appearance of onion neck collapse was consistent with that of Brazier buckling, with increasing ovalisation prior to failure at the site of catastrophic failure. At this site of failure, the neck crimp displayed an invaginated crease with a protrusion of deformed wall tissue at each end. Finally, the binary logistic regression model showed that the lodging status of onion plants could be predicted with an accuracy of 94% using the parameter, deformation at 4N transverse load against the recorded lodging status. Taken together, these finding indicate that changes in mechanical properties of the pseudostem tissues were a primary requisite

causing the reduction in structural integrity, which precipitated the buckling failure of pseudostems.

In contrast to other monocot species such as rice and wheat where ESWM is significant to stem lodging, the results from this study suggest that ESWM is not a principal regulator of canopy collapse in onion pseudostems. This is likely related to both the time at which true leaf blade senescence begins, and differences in plant architecture. While the onion canopy retains a high ESWM, the relatively even distribution of leaf mass along the psuedostems longitudinal axis would provide a lower moment of inertia compared to cereals where the centre of mass is much further from the rotational axis (Crook & Ennos 1994). Additionally, the moment of inertia in cereals increases during grain fill, while in onions this would appear to remain static until senescence, at which point it decreases. This, and the fact that ESWM did not decrease until later canopy collapse, provides sound evidence to support the hypothesis that onion canopy collapse is principally governed by 'softening' of the neck tissues.

During canopy collapse there was a net increase in plant total above-ground dry mass while chlorophyll content remained relatively high signifying that the crop was continuing to photosynthesize at least until 80% canopy collapse. In this study, there was a threefold increase in bulb dry matter and a reduction in foliage dry matter of 2.5-3g/plant suggesting that photosynthate and re-mobilised dry matter within the canopy favoured bulb over foliage growth during canopy lodging. Brewster et al. (1986) also noted a net loss of dry matter from

the foliage though it was to a lesser extent, and this discrepancy is likely due to the use of a different method when dividing plant structures, and the changes in that study were assessed from the initiation of bulbing. The steady decreases in foliage dry mass as assimilates were remobilised for use in bulb growth were accompanied by rapid reductions in foliar moisture content after the crop had reached 80% tops-down. This is consistent with field observations that the leaf blades were desiccating after canopy collapse. Desiccation was observed to start from the tips of leaf blades before progressing towards the sheath. The breakdown of chloroplasts has been described as the earliest and most significant change in plant cell structure during senescence (Lim et al. 2007), and in this study chlorophyll was retained in the youngest leaves despite the pseudostem collapse. A reduction in chlorophyll content of the youngest fully emerged leaf only occurred during the latter stages of the lodging period, and this is consistent with previous studies that have observed that the foliage does not senesce until after canopy collapse and bulbs have reached their final size (Brewster 2008). Data from this study and those previous support the hypothesis that photosynthesis continues during canopy collapse.

This partitioning of resources in favour of bulb growth is a key component of the stress avoidance adaptation of onion bulbs, with most of the increase in bulb dry weight completed by the time the crop had reached 80% tops-down. The timing of many of the significant changes in onion plant growth and development occurred at or after 80% tops-down, and included declines in foliage fresh and dry weight, foliage centre of gravity, chlorophyll content and an increase in bulb fresh and dry weight. This indicates that the onion plants were

completing their transition to bulb dormancy and may explain in part why the population development marker of 80% tops-down has been adopted in both the scientific literature (Mondal et al. 1986a) and commercial bulb production, despite limitations such as its binary nature and influence of outside factors such as wind.

The lodging of the pseudostem of onions could be induced at any stage of development by sufficient wind loading and a crop could be blown to 100% tops-down in a short space of time. However, the failure of a pseudostem which had not softened and hollowed would ovalise less and therefore longitudinal strain would not be reduced to the same extent as a more developed plant. Consequently failure by exceeding the longitudinal shear strength may occur, leading to tears in the outer layers of the pseudostem tissue and which provide a potential entry site for infection by pathogens. This would also be the case if an immature crop were pushed flat by machinery without clearance.

Deformation under transverse load can provide an objective indication of a plants developmental progress and when measured across multiple plants in a crop, provide an indication developmental uniformity within a crop population in a single visit, as opposed to a more difficult and subjective visual assessment of tops-down. This method has also proven to be relatively practical and robust, without the need for expensive, sensitive and fragile testing equipment. In the future, there may be scope to adapt the method to allow for non-destructive measurement of intact plants and the automation of sample analysis. This technique can also quantitatively assess variation in progress towards canopy collapse within

a crop. This is of importance as a consequence of plant-to-plant variation in development within a crop population as single pass systems lift bulb crops that are not entirely uniform in their development. Given the postulated relationship between development at lifting and bulb storage potential, homogeneity of crop development is desirable to achieve uniformity of bulb storage potential. Measuring developmental homogeneity could be used to optimise scheduling of crop lifting to meet the desired sizes, maximise potential yield and optimising quality when lifted in an indiscriminate single pass system.

This paper has presented evidence to support the hypothesis that lodging in onion bulb crops is primarily governed by plant development through 'softening' of the pseudostem. There is a clear increase in the deformation (ovalisation) under transverse load during the period leading up to pseudostem lodging. This evidence of tissue softening increases the probability of pseudostem lodging occurring, as gravity and wind act against the crop canopy to create angular momentum. The data supports the hypothesis that a developmental regulatory mechanism that controls foliage collapse is Brazier buckling of the pseudostem caused by a reduction in the transverse modulus of elasticity of the pseudostem. The binary logistic regression indicates that pseudostem deformation under transverse load can be used as a variable for the prediction of lodging and could be used by commercial producers to improve the scheduling of crop lifting. The mechanisms that control pseudostem softening were not a focus of this work, however mechanisms which have been shown to cause softening in ripening fruit such as cell wall structural carbohydrate breakdown (Brummell, 2006) are worthy of investigation as they are also related to development and changes to the rheological

properties of plant tissues. The role of non-structural carbohydrate in tissue osmoregulation may also warren investigation as this is also a key component of cell turgor.

The changes in deformation under transverse load appeared to be similar between the two crops monitored in this experiment. It has been understood for a long time that the maturity of onion bulb crops is determined by the genetics of the cultivar grown interacting with the day length and temperature in the environment in which it is grown. It would therefore be pertinent to measure the differences in the newly developed deformation under transverse load method of tracking maturity among a range of cultivars. This is the focus of chapter 5.

Chapter 5: A comparison of pseudostem lodging among onion (*Allium cepa* L.) cultivars

Abstract

Lodging of pseudostems is a natural part of onion ontogeny. Lodging occurs during the latter stage of bulb growth and is used in commercial crop production and scientific studies as a key indicator of plant development. There is evidence that plant regulation of pseudostem lodging is governed by the softening of the base of pseudostems. Whether softening of the pseudostem base leading to canopy collapse differs among cultivars is unknown. To evaluate this, pseudostem deformation under transverse load was measured during the latter stages of plant development in the lead up to and during lodging in hybrid cultivars '*Baron'* and '*Rhinestone',* and open pollinated cultivars *'Early Creamgold'* and *'Regular Creamgold*'. Pseudostem deformation was similar among cultivars when compared at the same stage of development, albeit pseudostem collapse was delayed in *'Regular Creamgold*'. Estimated Self-Weighted Moment (ESWM) differed among cultivars but was not the governing mechanism of lodging. Softening of the base of the pseudostem was found to be the governing mechanism of pseudostem collapse independent of genotype

Introduction

The time of lifting of onion crops relative to canopy collapse has been shown to affect bulb yield, quality and storage life (Chapter 3). An investigation by the author has demonstrated that softening of the base of pseudostems can be used to predict the timing of canopy collapse for only two cultivars ('*Regular Creamgold*' and '*Plutonus*') planted at different times at different sites (Chapter 4). However, there has been no comparison of the pseudostem mechanical properties which govern pseudostem collapse among cultivars grown in the same environment, although photothermal requirements for canopies to collapse differs among cultivars (Lancaster et al. 1996).

In cool-temperate climates, the stage of development at which bulbs are lifted from the soil before curing has been shown to affect gross yield, marketable yield and storage potential of onion bulbs (Chapter 3 ; Komochi 1990; Sargent et al. 2001; Wright et al. 2001). The stage of onion crop development at lifting is therefore an important management factor. It is common practice in commercial onion production in temperate regions to lift crops when between 50 to 80% of plants within a population have lodged. This has also been used as a marker for development in studies of plant physiology (Brewster 2008; Chapter 3 ; Gubb et al. 2002; Mondal et al. 1986a). Although widely used as an indicator of crop development, this approach has limitations as lodging may be caused by external factors such as wind loading rather than development *per se* (Berry et al. 2006; Heath 1945). Importantly, from an operational perspective, the ability to predict when the crop will reach the optimum development for lifting (generally thought to be when the canopy reach 80% tops-down) is

fundamental to scheduling the lifting of crops in temperate environments. The inability to do this poses a significant limitation to the reliable production of robust bulbs suitable for export markets (Chapter 3).

Alternative or complementary markers for monitoring bulb development that are independent of environmental factors and that assist in predicting canopy collapse for scheduling lifting of crops are needed. A greater understanding of the underlying processes involved in bulb development and pseudostem tissue failure may contribute to improved predictions. Pseudostem lodging is a part of an onion's life strategy and accompanied with the senescence of leaf blades and roots, and the bulb's transition to dormancy (Rey et al. 1974). Work undertaken by this author (Chapter 4) has shown that chlorophyll content declined within the youngest leaf blade and foliage moisture content decline rapidly at the time of lodging consistent with the finding of (Heath 1945) and has added to the knowledge of canopy collapse by quantifying the softening of the base of the pseudostem. This softening of tissue at the base of the pseudostem through deformation under transverse load was investigated as an alternative method (Chapter 4). However, the work was based on two cultivars only with limited understanding of the mechanical properties of pseudostems.

The author has not found any quantitative studies of the mechanical properties of onion pseudostems, however a qualitative description by Heath (1945) attributed canopy collapse to the pseudostem forming into a thin walled hollow tube after the last leaf blade emerged. Alternatively, Mann (1952) described the destruction of parenchyma cells which precipitated

canopy collapse in garlic as starting from the adaxial epidermis until the outer epidermis, vasculature tissue and a few parenchyma cells remain. Mann (1952) specified that this differed from the description of pseudostem collapse provided by Heath (1945) and recommended further investigation. Examination of the mechanical properties of pseudostems and how they change over the period leading up to lodging may help to clarify the changes described by previous authors.

The mechanical properties of plant tissue in other species have been shown to be a function of tissue water relations and tissue structure and composition (Niklas & Spatz 2012). Cell turgor has been linked to the compressive resistance of tubular plant structures subject to a bending moment (Schulgasser & Witztum 1997) and therefore changes in turgor are a possible cause of structural weakness in pseudostems, especially where a reduction in resistance to compression leads to Brazier buckling. In *Alliums*, Niklas and O'Rourke (1987) found that the flexural rigidity of chive (*Allium Schoenoprasnum* var. schoenoprasnum L.) leaf blades was a function of water potential (Niklas et al. 1987), suggesting that compressive failure may be due to a reduction in turgor. Yet, pseudostems of onions collapse despite abundant soil moisture and turgid leaf blades, although the process is hastened by drought (Heath 1945) indicating that turgor control may play a role in some circumstances.

Pre-tensioning of the epidermis is understood to develop under positive turgor where circumferential expansion is constrained, and contributes to the mechanical integrity of herbaceous structures (Schulgasser & Witztum 1997). Little is known as to the role of

circumferential tension in the structural integrity of onion pseudostems. Some plant structural failures including cracking in the roots of carrots (Gracie 2002) and hollow stem cavity formation in broccoli (Boersma 2009) have been attributed in part to high levels of circumferential tension. As the pseudostems of onions are observed to soften during the period leading up to lodging it is posited that there would be a reduction in circumferential tension during this period, contributing to structural failure of the pseudostem.

Though it has long been understood that cultivars have different day length and thermal time requirements in order to reach maturity there has been no comparison of the pseudostem mechanical properties among cultivars grown in the same environment. This study addresses this paucity in understanding by quantifying the mechanical properties of pseudostems as an alternative method to a visual assessment of tops-down among cultivars and by elucidating the mechanics governing canopy collapse in onions. We hypothesize that the softening of the pseudostems of onions which governs their collapse is the same among onion cultivars when compared at the same stage of development.

Materials and methods

Two experiments were conducted in season 2013/14 to investigate phenotypic variation in pseudostem lodging and the mechanical properties associated with the lodging process. The first experiment considered the differences among four cultivars in the softening of the pseudostem base and canopy collapse. The second experiment focused on the change in mechanical properties.

Cultivar comparison

Phenotypic variation in pseudostem lodging was investigated using four commercial cultivars that included two F1 hybrids, *'Baron'* and *'Rhinestone',* and two open pollinated cultivars, *'Early Creamgold'* and '*Regular Creamgold*', in an experiment at the TIA Vegetable Research Facility, Devonport, Tasmania, Australia. F1 hybrid and open pollinated cultivars were included to provide a range of material relevant to the Tasmanian production system. The intent was not to contrast hybrid and open pollinated cultivars. The four cultivars were planted on the 9th of September 2013 in a randomised complete block design comprising four blocks. A plot comprised a single bed (2m wheel centres) 20m long that was direct seeded in 10 rows with a target population of 70 plants/m^2. A block comprised four adjacent beds. Each cultivar was randomly assigned to one of the plots (beds) in each block and cultivated in accordance with standard commercial practice. A section of a row containing 20 plants within each of the 16 plots (4 cultivars by 4 blocks) was marked and the number of plants that had lodged was assessed twice per week. Daily minimum and maximum temperatures were recorded using a nearby weather station for the calculation of thermal time.

Four plants were randomly sampled from each plot at predawn during the latter period of bulb expansion through to 100% tops-down. After sampling, soil was wiped from each bulb and roots removed. One plant from each plot was used for quantification of pseudostem deformation under transverse load and circumferential tension. The other three plants were used to determine fresh mass of bulb and foliage, and the location of the foliage centre of gravity.

Water relations and mechanical properties

Association between the mechanical properties, water relations and lodging were investigated in a commercial crop (CGa14) located near the town of Hagley, Tasmania, Australia. The crop (‘*‘Regular Creamgold,’*) was planted on the 12th of September 2013 on preformed beds, with a target stand density of approximately 70 plants/m^2. Seeds were sown in a 10 row configuration on 1.8m wheel centres. A 5m length of bed was selected for this study. Plants were randomly selected and removed at regular intervals from this section of bed from mid-bulbing until the canopy of all plants in the section had collapsed. Six plants were randomly sampled prior to dawn at each sample date. Three plants were used to measure osmotic potential in the pseudostem and bulbs. The other three plants were used to measure gap index and deformation of the neck under transverse load. The proportion of plants with lodged canopies was recorded in five linear metres of bed adjacent to the selected sampling area.

Foliage and bulb mass

Foliage was separated from bulbs immediately after hand harvest by severing at the point where the pseudostem widens to form the bulb. Mean bulb and foliage fresh mass for each plot were recorded.

Estimated self-weight moment

The location of the foliage centre of gravity was determined using the method employed by Crook and Ennos (1994). After severing from the bulb as described above, the foliage centre of mass was determined by balancing the foliage on a thin straight edge (2 mm width). The distance of the pivot point from the pseudstem base was considered an estimate of the location of the centre of gravity. This value was used to determine the estimated self-weight moment (ESWM) at 30° and calculated using the formula:

$$ESWM = sin\theta hmg$$

where θ is the angle relative to vertical, h is the height of the centre of gravity, m is the mass of the pseudostem, and g is the acceleration constant due to gravity (Crook & Ennos 1994). .

Pseudostem mechanical properties

Pseudostem deformation (ovalisation) under transverse load was recorded using the method described in Chapter 4 (Page-80). In short, a transverse section 15 mm long was excised from the base of the pseudostem and placed between two polyethylene plates (11 mm thick). A lever was attached to one plate and a force gauge was mounted on the opposing plate. A force of 0, 4, 8, 12 and 16 N was applied perpendicular to the longitudinal axis of the pseudostem. When sections were not round (i.e. already displayed some degree of ovalisaton), the segment was oriented so that the shortest axis was perpendicular to the two plates. This orientation ensured that the force applied was parallel to the plane in which failure would normally occur. Plan view images were taken using a digital camera mounted above the specimen and used to determine the degree of ovalisation at each transverse load.

Gap index

Immediately after sampling, residual strain was estimated by excising a transverse segment 5 mm long from the base of the pseudostem. Sections were placed in a Petri dish and a radial incision was made from the centre to the peripheral layer. Digital images were then taken using a digital camera (Cannon PowerShotA530) mounted above the dish and the gap index as an indication of circumferential tension (Sorensen et al. 2000) was calculated using:

$$I_g = \frac{w}{(d_1 + d_2)/2}$$

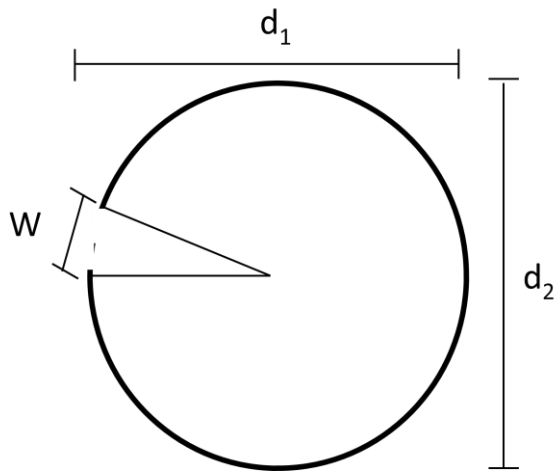

Figure 5-1. Diagram of Gap index measurement of onion pseudostem segments

Where W is the width of the gap and the width of the section measured perpendicularly is denoted by d_1 and d_2 All distances were measured using ImageJ (Version 1.44p, National Institutes of Health, USA) software calibrated to the images using graph paper of a known dimension.

Thermal time

The cumulative thermal time (Day Degrees) to 80% tops-down was calculated as:

$$GDD = \sum_{d=0}^{d=80\%\ tops-down} \frac{\mathrm{Tmax} - \mathrm{Tmin}}{2} - \mathrm{Tb}$$

; Where Tmax and Tmin are the daily minimum and maximum temperatures, d the number of days after planting, and the base temperature Tb=6 °C as used by (Tei et al. 1996)

Osmotic potential

Tissue was excised from each fleshy layer of leaf or blade tissue at the base of the pseudostem and from each layer at the equator of the bulb and placed in separate 1.5 ml centrifuge tubes and frozen (liquid nitrogen or dry ice) in the field immediately after sampling. The osmotic potential of each tissue layer from the pseudostem and the bulb was determined in a laboratory by first thawing and centrifuging (Eppendorf 5424 microcentrifuge) for 10min at 20238RCF. A 10 µl sample of the supernatant was then transferred to an Osmometer (Wescor Vapro 5520, Logan, UT, USA) for quantification. The Osmometer was calibrated using NaCl standards (mmol kg^{-1}) with readings converted to MPa. The mean value across all layers at the sampling region of each individual bulb was used in the analysis.

Statistical analysis

Statistical analyses were performed using SPSS (ver. 22). Data from the cultivar comparison trial were analysed using a General Linear Model. Cultivars were compared at a given number of days after planting and at the same developmental stage as determined by tops-down %. Assumptions of normality and homoscedasticity were assessed and data transformed where necessary to meet these assumptions. Binary logistic regression was

performed to determine the relationship between the degree of softening (as measured by deformation under 4N transverse load) and the probability that the assessed pseudostem had lodged across all cultivars. In the water relations and mechanical properties experiment, Mixed Model analyses were used to test the effect of the fix factors; sample date and position and their interactions on tissue osmotic potential. position within plant was included as a random factor to test the ‘position’ effect and the residual was used to test all other effects. Spearman's correlation was performed to investigate the relationship between gap index and deformation at 4N transverse load.

Results

Cultivar comparison

The time taken for the plant population to reach 80% tops-down in the cultivar comparison was similar (132-133 days after planting) for cultivars *'Baron', 'Rhinestone'* and *'Early Creamgold'*. Of these, lodging was most uniform in cultivar 'Rhinestone', with 90% of plants lodging over a three day period. Comparatively, cultivar *'Regular Creamgold,* was slowest to lodge and did not reach 80% tops-down until more than 146 days after planting. The difference between the *'Early Creamgold'* and *'Regular Creamgold,* cultivars reaching 80% tops-down equated to 167 growing day degrees (**Table 5-1**).

Table 5-1. Number of days and thermal time (Growing Day Degrees) from planting to 80% tops-down for each cultivar, base temperature Tb=6°C (Tei et al. 1996).

	DAP to 80% tops-down	**GDD to 80% tops-down**
'Baron'	133	1185
'Rhinestone'	132	1173
'Regular Creamgold'	146	1341
'Early Creamgold'	132	1173

The fresh mass of bulbs increased in all cultivars by approximately 50% from just prior to commencement of tops-down to the end of the sampling period. This equated to a mean increase in bulb fresh mass of 2.8 $g.day^{-1}$ (**Figure 5-2**.b). Foliage mass declined over the same time frame. At 122 days after planting, *'Regular Creamgold'* had the heaviest fresh

foliage mass at 83g per plant and *'Early Creamgold'* the least at 40g per plant ($F_{3,9}$=7.58, p=0.01) (**Figure 5-2**.b). However when analysed at the same stage of development (approximately 80% tops-down '*Baron'*, *'Rhinestone'* and *'Early Creamgold' 133 DAP, 'Regular Creamgold' 148 DAP*), foliage mass per plant did not differ among cultivars ($F_{3,9}$=2.66, p=0.15). During the six days prior to lodging both the foliage fresh mass and centre of gravity either remained constant or declined. The location of the foliage centre of gravity was similar among cultivars when compared at similar stages of tops-down ($F_{3,9}$=0.01 and P =0.928). From commencement of lodging to 80% tops-down the ESWM declined largely as a consequence of declining foliage fresh mass. The centre of gravity did not decline until after 80% tops-down (**Figure 5-2**d).

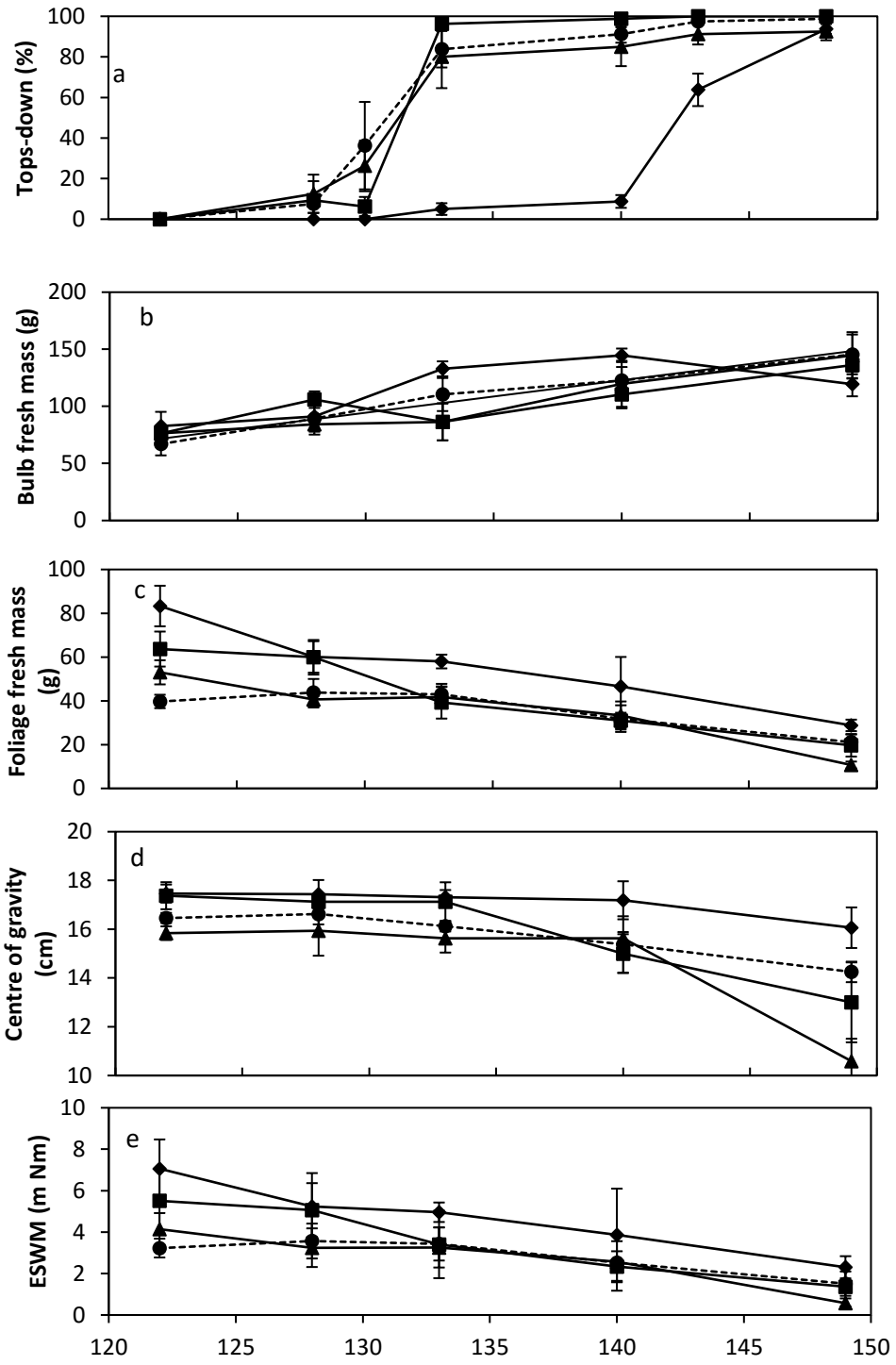

Figure 5-2. Plant mass over the latter stages of bulbing; tops-down percentage (a), bulb fresh mass (b), foliage fresh mass (c), centre of gravity (d), estimated self-weight moment at 30° (e). for cultivars *'Baron'*(▲), *'Rhinestone'*(■), *'Early Creamgold'*(●) and *'Regular Creamgold'* (◆). Error bars indicate ± 1 SEM, n=4.

Pseudostem resistance to deformation decreased over the assessment period in all cultivars (**Figure 5-3**b). The degree of deformation was greatest in '*Regular Creamgold'* when compared at the same stage of development (approximately 80% tops-down), ($F_{3,9}$=5.00, p=0.025) (**Figure 5-3**b). This difference in sensitivity to deformation may have been due to deformation assessments of '*Regular Creamgold'* at >90% tops down. All cultivars reached a deformation index of at least 0.4 under four newtons transverse load at 80% tops-down. Mean Gap Index not different among cultivars at 140 days after planting when differences in tops-down % were greatest ($F_{3,9}$=1.97 , p=0.19) but did differed with time after planting ($F_{4,48}$=7.16, p<0.001).

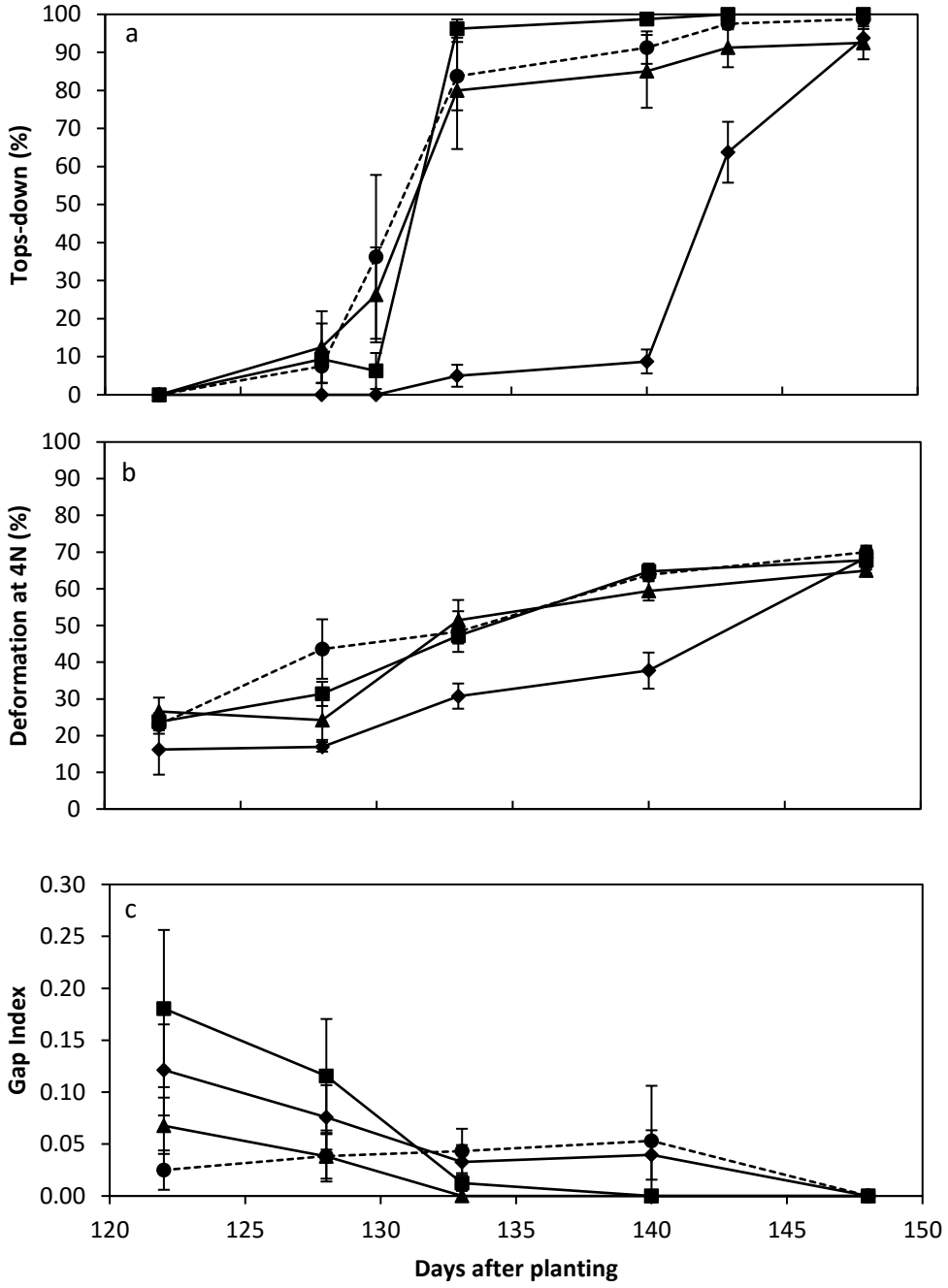

Figure 5-3. Pseudostem properties; tops-down percentage (a), Deformation of pseudostems under transverse load (4N) (b), Gap Index(c) for each *Baron'*(▲), *'Rhinestone'*(■) and *'Early Creamgold'*(●), *'Regular Creamgold'* (◆)over the latter stages of bulbing form 122-148 days after planting. Error bars indicate ±1 SEM, n=4

There was a weak, negative correlation between deformation of pseudostem segments with 4N transverse load and the gap index (Spearman's r=-0.476, p<0.001) (**Figure 5-4**). Both of these factors also changed with time after planting.

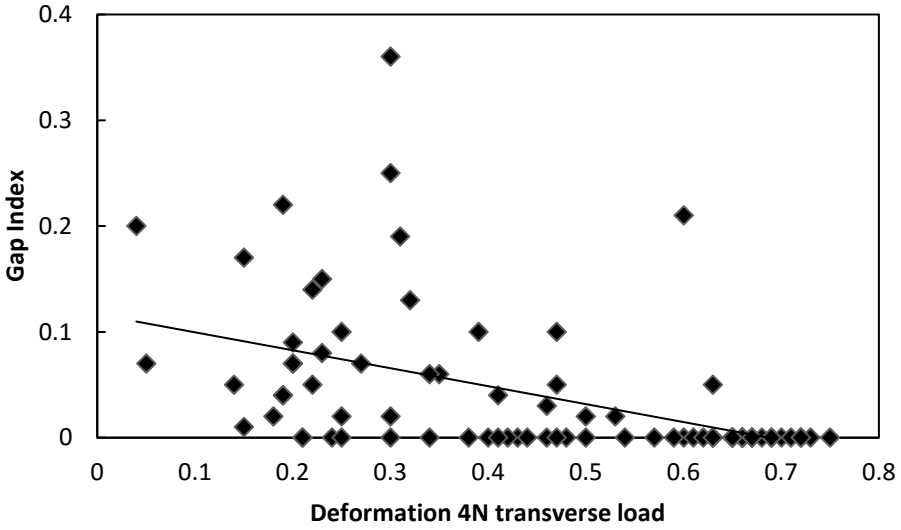

Figure 5-4. Gap index and Deformation at 4N transverse load of *'Regular Creamgold' 'Baron', 'Rhinestone'* and *'Early Creamgold'*.

Logistic regression

Binomial logistic regression was used to evaluate the predictive value of deformation at 4 Newtons transverse load on the probability of pseudostem lodging across genotypes. The model;

$$Logit(p) = Deformation\% * 0.303 - 12.588$$

was statistically significant (χ^2 =87.9, N=80, P<.001) with 89% (Nagelkerke R Squared) of the variance in lodging status explained. The model which correctly classified the lodging status of 92.5% of plants. The probability of a pseudostem lodging was 50% when the deformation was equal to 42%.

A clear differentiation of development was possible based of the deformation at 4N transverse load independent of genotype and prior to the start of canopy collapse. All cultivars displayed a similar pattern of increased pseudostem deformation when additional load was applied, however beyond 4N load the deformation increased did not aid the discrimination among groups (**Figure 5.5**b).

At 133 days after planting 4 Newtons of load produced the greatest difference in deformation between the *'Regular Creamgold'* cultivar, which had not lodged, and the other cultivars, which had reached 80% tops-down. There was no difference in the deformation among cultivars at 148 DAP when all plants had lodged (**Figure 5-3**).

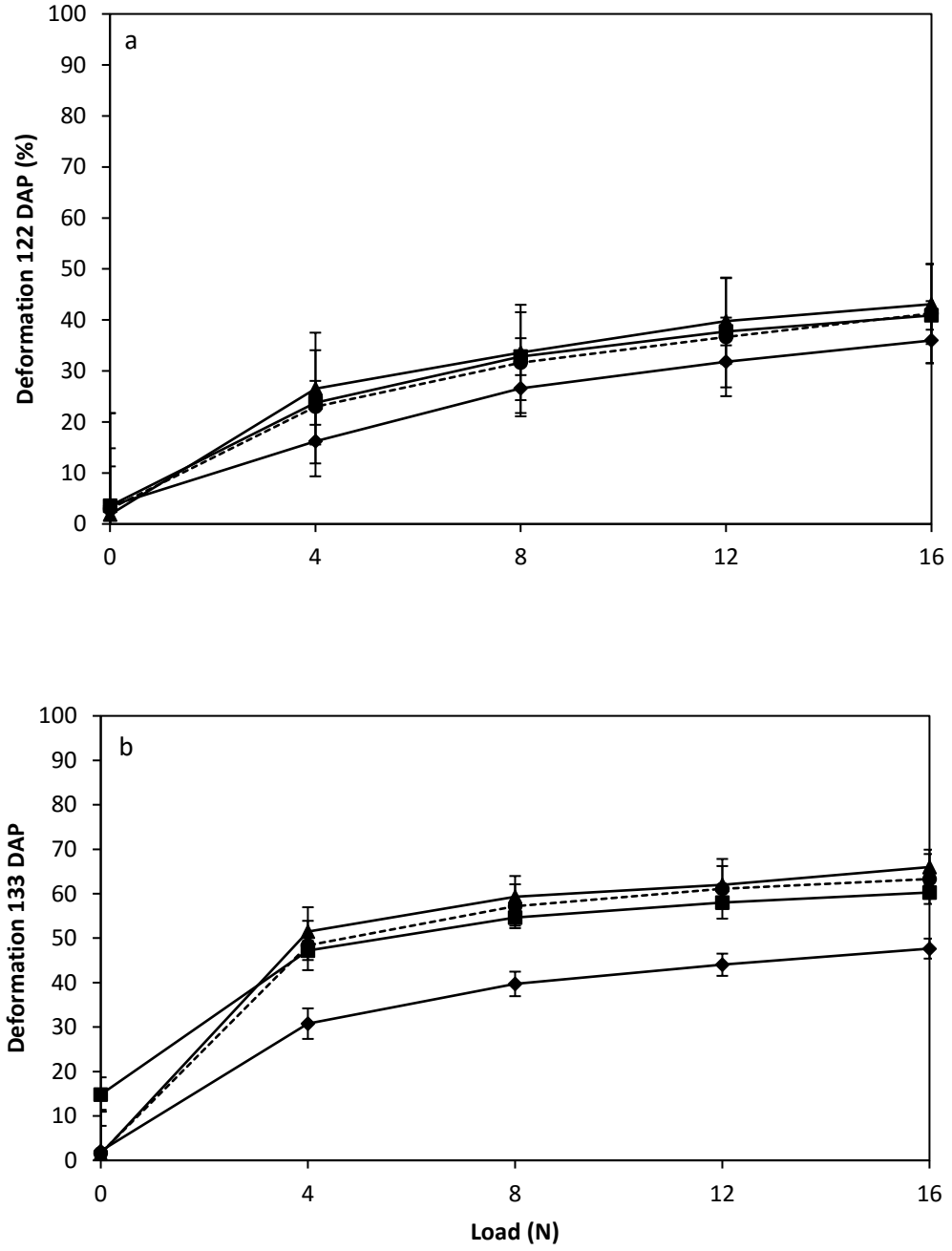

Figure 5-5. Pseudostem deformation under applied load of 0, 4, 8, 12, 16 Newtons transverse load at 122 DAP (tops-down 0% across all cultivars) (a), 133 DAP (tops-down> 80% for *'Baron'*(▲), *'Rhinestone'*(■) and *'Early Creamgold'*(●), *5% tops-down 'Regular Creamgold'* (◆))(b). Error bars indicate ± SEM, n=4.

Water relations and mechanical properties

The osmotic potential of the pseudostem and bulb tissue was not significantly different ($F_{1,14}$=0.003 p=0.955) at any of the sampling dates (**Figure 5-6**). When averaged for both pseudostem and bulb tissue, the mean osmotic potential decreased from -0.4 MPa at early bulb initiation (118 DAP) to -0.75 MPa at commencement of tops-down (141 DAP), and continued to decline to -0.9 MPa at 95% tops-down (152 DAP). Similarly, the Gap Index decreased from 0.07 at early bulb initiation to 0 at 95% tops-down (**Figure 5-6**). An increase in the deformation at 4N transverse load preceded tops-down. Deformation under transverse load of 4N showed a gradual linear increase of 0.6% per day over the period from 118 days to 141 days (**Figure 5-6**.b). Over the following 11 day during which the canopy collapsed, deformation increased to 0.5.

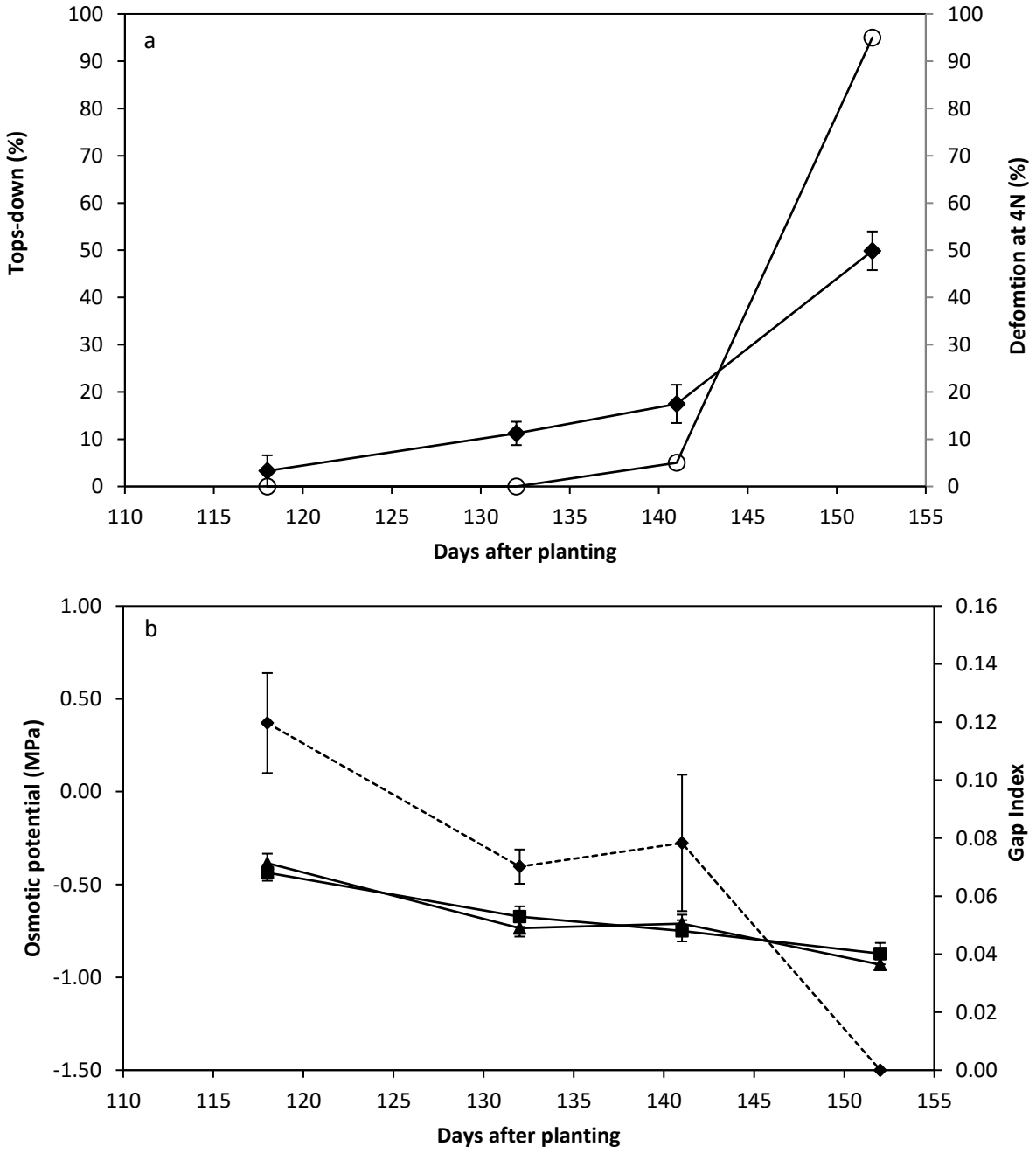

Figure 5-6. Tops-down percentage(○) and deformation at 4 Newtons transverse load (◆) (a), Gap Index at dawn (◆) and osmotic potential (MPa) of pseudostem(■) and bulb(▲) tissue (b) during the latter stages of bulbing of '*Regular Creamgold'*. Error bars indicate ± SEM, n=3.

Discussion

The development stage at which onion crops are lifted has been shown to affect both yield and bulb quality (Chapter 3 ; Nilsson 1980; Suojala 2001). An alternative or complimentary indicator of onion plant development would assist scheduling of onion crop lifting based on development to optimise bulb storage quality. In this study, the phenotypic variation in lodging among genotypes was investigated to validate the use of pseudostem softening as a marker of plant development. Differences in plant development were apparent in the softening of tissue at the base of the pseudostem before any of the plants in the sample population had lodged. Therefore tissue softening at the base of the pseudostem provided an indicator of plant development through time independent of genotype. Pseudostem softening, in conjunction with other indicators of onion plant development (Gubb & MacTavish 2002; Lancaster et al. 1996; Mondal et al. 1986a; Sargent et al. 2001; Wright & Triggs 2005), could be used for monitoring a crop's progression toward optimal lifting maturity. This is the first published comparison of pseudostem softening among cultivars.

Lodging in cereal species has been attributed to increasing ESWM commensurate to development, yet in onion, a monocot with a different architecture, this was not the case. Of the four cultivars monitored, *'Regular Creamgold'* had the highest ESWM but lodged later than the other three cultivars. This cultivar is usually planted before mid-September therefore was the only cultivar planted beyond its normal planting window. Thus, the delayed lodging was most probably due to its greater photothermal requirement. However 'Regular Creamgold' did collapse earlier due to its higher ESWM. Crook and Ennos (1994) showed

that differences in lodging susceptibility among wheat cultivars was governed by differences in ESWM, root anchorage and stem strength. In onions, differences in lodging among cultivars were not primarily governed by ESWM but, instead, softening of the pseudostem tissue. While the moment of canopy collapse is provided by the mass of the foliage (and gravity), in the absence of psuedostem tissue softening, this did not lead to structural failure. This reinforces the findings of Chapter 4 where the softening of the base of the pseudostem was identified as the governing mechanism for canopy collapse within onion crops. Because ESWM is not a primary determinant of canopy collapse, it is unlikely that it would explain difference in the timing of canopy collapse among cultivars.

The disadvantages of using tops-down percentage as an indicator of plant development is change may be slow or rapid, and affected by wind making it difficult to predict. The rate of change is dependent partly on the uniformity of crop development with individual, plants are either erect, lodged or for a short period “lodging”. This may have contributed to the range of recommendations in the literature for lifting scheduling lifting based on tops-down (Brewster 2008; Brewster et al. 1986; Gracie 2006; Komochi 1990; Mondal et al. 1986a; Nilsson 1980; Suojala 2001; Wright et al. 2001). The gradual increase in deformation under transverse load, a continuous measure, as plants progressed towards tops-down was consistent among all cultivars. These properties make tissue deformation a preferable marker of onion crop development for scheduling lifting, in contrast to the binary nature of canopy collapse.

The relationship between deformation under transverse load measurements and the probability of onion crop lodging may assist with the optimising of crop lifting. Prior to the investigation carried out in chapter 4 there this relationship had not been described. In this study binary logistic regression correctly classified 92% of cases when the data from all of the cultivars was combined, based on the deformation at four newtons transverse load. This was similar to (Chapter 4) where 94% of cases were classified correctly. This indicates that the method of measuring deformation under transverse load in order to objectively determine development is valid across a range of genotypes and would not require a separate model for each cultivar.

Bulbs were highly turgid and expanding rapidly while the pseudostem softened, leading to structural failure. The reasons for the differences in the mechanical properties of bulbs and the base of the pseudostem are unclear however previous studies have highlighted the role of water relations in chives (Niklas & O'Rourke 1987). The osmotic potential decreased over time in both the neck and foliage in the commercial crop and were not significantly different. These osmotic potentials were similar at tops-down to the osmotic potential of bulbs evaluated in previous studies (Sinclair et al. 1995). The decrease in osmotic potential was not a consequence of crop water deficit as the crop continued to be watered on a regular basis. Given that the water potential of the plant would have been in equilibrium with the soil at predawn when the samples were taken it is reasonable to assume that tissue turgor would have been increasing as the pseudostem softened. Rapid bulb expansion during this phase is indicative of high bulb tissue turgor potential. There is clear evidence that pseudostems soften

during the lead up to collapse provided by the increase deformation under transverse load and reduction in residual strain. This appears to be a consequence of changes in the mechanical properties the pseudostem tissue.

Pre-tensioning of the epidermis is considered to contribute to the mechanical integrity of herbaceous structures (Niklas et al. 1997). This pre-tensioning of the epidermal tissues is understood to develop under positive turgor where circumferential expansion is constrained (Schulgasser & Witztum 1997). Circumferential residual strain measured in this study did decline prior to lodging, suggesting that the reduction may contribute to the structural failure of the pseudostem. However circumferential residual strain did not differ among cultivars when tops-down % did and therefore only a partial association with structural failure of onion pseudostems. Plants may be at little risk of lodging when circumferential tension is high, as this indicates the structure is likely to be turgid. When the circumferential tension is close to 0 the plants may be at an increased risk of lodging, however a gap index of 0 does not indicate that plant lodging has occurred. Thus indicating that additional softening of tissues at the base of the pseudostem precipitate structural failure after the gap index reached 0. Gap index as an estimate of residual circumferential strain appears not to be a suitable indicator of plant development for the forecasting of lodging. In conclusion, softening of the base of the pseudostem is a key developmental change which regulates lodging across cultivars. The softening of onion pseudostems was similar among cultivars when evaluated at the same developmental stage and therefore appears to be a valid marker of plant development for the scheduling of crop lifting. Despite clear differences in tissue mechanical properties the

osmotic potential of pseudostem and bulb tissue did not differ at each sampling time and declined during the period from early bulbing to lodging. Softening of pseudostems could be used as an indicator of plant development for the scheduling of onion crop lifting independent of genotype.

Chapter 6: General discussion

This industry-focussed study was undertaken to address knowledge gaps relevant to the management of Tasmanian onion production and postharvest handling for the reliable supply of European markets. Specific knowledge gaps addressed include the timing of onion crop lifting, the mechanisms governing pseudostem collapse, and the effect of mechanical impacts on bulb storage life and yield. This chapter discusses the key thesis findings in the context of the existing literature and provides practical recommendations for the benefit of the Tasmanian onion industry.

In this thesis elements of onion bulb production and handling systems were examined based on their potential influence on yield and storage potential. The timing of crop lifting was identified as a key agronomic practice and investigations subsequently related pseudostem lodging (a developmental marker for lifting) to yield and storage life. Lodging (tops-down) was confirmed as an important developmental indicator of the optimal time to lift. The mechanism governing this process was investigated and identified pseudostem softening as the primary mechanism governing collapse. This softening was shown to have potential as a predictive tool to facilitate improved scheduling of crop lifting. As growth rates prior to and during bulbing may influence the timing of canopy collapse, the effect of high nitrogen and defoliation during the vegetative stage was investigated. These growth manipulation treatments reduced portioning to the bulbs, reducing yield. High nitrogen rates also produced softer bulbs, a trait associated with reduced storage potential. This study has shown the

importance of pre-harvest factors as underpinning a bulbs maximum storage potential, which can then be reduced by post-harvest practices. The handling of bulbs post-harvest was first investigated by monitoring the storage of bulbs from several crops sampled from different locations on a commercial grading and packing line. As the packing line was shown to affect the storage potential of bulbs, controlled impact studies were undertaken to better understand the effect of magnitude and location of impacts to the surface of the onion bulb. These studies confirmed results from earlier studies (Herold et al. 1998; Timm et al. 1991) highlighting the important negative influence of impacts on storage quality, and also revealed that impacts to the base of the bulb are of much greater importance than blows to other surface areas .

When to lift crops?

In this study, onion storage life was maximised by lifting crops at *ca.* 90% tops-down (Chapter 3), this being slightly later than the current industry standard of 80% canopy collapse. Lifting bulbs earlier or later than this stage of crop development resulted in reduced bulb storage life due to increased internal sprout growth and higher respiration rates associated with both maintenance and growth. Although current commercial practice is to lift crops when they reach 80% tops-down, the slightly later lifting at 90% provides an opportunity to increase yield and maximise bulb storage life in Tasmanian cropping systems. Field staff may need to increase their monitoring of crops during tops-down in order to schedule crop lifting for this stage of development and some compromise of these schedules may be required depending on the variability of tops-down within each crop.

Onion bulbs naturally transition from growth and development to dormancy. During this transition leaf blades and pseudostems senesce while bulbs continue to gain weight (Brewster 2008). As crop lifting is a well-recognised disruptive agronomic intervention used in temperate environments during this transition, the timing of lifting on bulb yield and quality has received much attention (Brewster 2008; Komochi 1990; Nilsson 1980; Sargent et al. 2001; Suojala 2001; Wright et al. 2001). However, results from these studies have been inconsistent. Ostensibly, this is due to the interaction of genotype and environment with lifting time. To the knowledge of the author, this is the only study to have assessed the link between time of lifting and long-term (>3months) storage of onions at *ca* 20°C across multiple crops and seasons. In this study, lifting at *ca* 90% tops-down was determined as a consistent developmental optimum (Chapter 3) across several onion cultivars, locations and years. This finding is later than the recommendations of Wright et al. (2001) who stored bulbs for up to 3 months, and subsequently recommending 60-80% tops-down to minimise disease incidence and maximise skin quality. Lifting at 90% tops down is also within the upper range of 50- 100% tops-down recommended by Suojala (2001) to maximise yield and minimise bulb sprouting. This suggested range is broad, and its breadth is most probably related to the caveat that there is not a rainy climate which may encourage disease and bulb storage at 1°C, with this low temperature contributing to depressed metabolic activity and low disease activity resulting in improved storage life. The differences in the findings of this study and others is likely a consequence of differing local practices, driven by climate, farming practices, logistics and market destinations. In comparison with the recommendation

of other studies, the finding of this study provides better alignment with Tasmanian industry practice aimed at achieving long term ambient storage.

The lowest internal sprout growth and respiration rate were observed in bulbs lifted at *ca* 90% tops-down, indicating that dormancy signals may be strongest when bulbs are lifted at this stage of development. While other studies have found an association between sprout growth and respiration rate (Yasin & Bufler 2007), this study also demonstrated a linkage between these and the timing of lifting. One possible explanation for the effect of time of lifting on respiration and sprout growth rates, these effectively representing bulb dormancy, is that interrupting plant growth and development at *ca* 90% tops-down maximises the expression and/or transfer of sprout inhibiting compounds from senescing foliage to bulbs. Previous authors have postulated the existence of inhibitory compounds in onion leaves, with these compounds translocating to the bulb to inhibit sprout growth during foliage senescence (Stow 1976). While this study was able to show defoliation increased sprouting, the presence of the proposed inhibitory candidates was not directly associated with sprouting. Abscisic acid (ABA) has also been investigated as a potential sprouting inhibitor during onion leaf senescence (Chope 2006), however applications of ABA analogues did not affect sprouting. This study provided some circumstantial evidence that inhibitory compounds may exist. Previous studies have not established a direct causal relationship between these substances and the inhibition of onion sprout growth (Stow 1976). This is possibly due to the complexity of mechanisms involves and the lack of genetic uniformity within many onion populations. Future studies could take advantage of recent advances in metabolomics, proteomics and

transcriptomics to unravel this ambiguity by providing a thorough description of these process.

Lifting before full canopy senescence ultimately compromises gross yield, however net yield is a function of both gross yield and quality. Ultimately there is a nexus in the trade-off between gross yield and quality to maximise net yield. Thus understanding the pattern of yield accumulation and the effect of lifting time on quality is paramount. Similar harvest trade-offs are made for a range of crops and indicators used to optimise harvest timing such as a threshold brix level for apples (Peirs et al. 200) tenderometer reading for vining peas (Martin 1981) and dry matter content in sweetcorn (Wong et al. 1994). Onion however appear to be relatively unique in being optimised by the timing of the structural failure of the pseudostem. This study confirms earlier reports that bulb mass increases by 30-40% from the beginning of foliage collapse to complete leaf blade senescence in undisturbed bulbs (Brewster 1990). Here, 25 to 40% of the final crop yield occurred from the commencement of canopy collapse to 2-weeks after tops-down. The increase in yield during late crop development is likely due to continued photosynthesis and mobilisation of carbohydrates and other assimilates (e.g. amino nitrogen) from foliage to expanding bulbs (Nilsson 1980) during senescence. This was evidenced by the retention of functioning chloroplasts (Chapter 4) in the youngest leaves, which only declined after the crop had begun to lodge, supporting Brewster's (1990) hypothesis that photosynthesis in addition to re-assimilation may still make a significant contribution to bulb mass accumulation during the early stages of canopy collapse.

The limitations of tops-down as a marker for predicting development were apparent with some crops reaching target tops-down percentage before or after the date predicted by experienced field officers. The combination of the agronomic importance of scheduling lifting (Chapter 3) and the absence of other clearly defined markers immediately prior to tops-down have made predicting development a key challenge for the onion export industry. Thus it became apparent that quantifying the governing mechanism of canopy collapse would be beneficial the scheduling of crop lifting.

What governs canopy collapse?

Softening of the pseudostem base was identified as the developmental event governing lodging in onion crops, with this change in tissue viscoelastic properties precipitating failure through Brazier buckling. (Chapter 4 and 5). Prior to this study only observational descriptions of pseudostem softening had been published (Heath 1945; Rey et al. 1974). The new mechanistic understanding elucidated here can be used to improve the scheduling of crop lifting to optimise yield and storage life. This is a significant contribution to the scientific understanding and practical management of onion crop development.

In contrast to cereals where canopy collapse is predominantly governed by increasing self-weighted moment, lodging in onion pseudostems was primarily governed by tissue softening leading to Brazier buckling at the base of the pseudostem. While psuedostem softening was judged the primary governor, estimated self-weighted moment was thought to play a

secondary role, providing the rotational force (moment) needed to create failure through Brazier buckling once the neck tissues had weakened. This contrasts with other crops such as wheat and rice where the increase in ESWM led to structural failure of the stem (Crook & Ennos 1994; Moldenhauer & Moldenhauer 1994) or root lodging. Although cultivars varied in their ESWM, canopy collapse in all genotypes occurred only after the tissue softened at the base of the pseudostem, further supporting the notion that softening is the primary condition governing canopy collapse in onions.

Brazier buckling in onion pseudostems requires failure of multiple sheath tissues on the side of the neck undergoing compression (Schulgasser & Witztum 1997), with this failure resulting in invagination of tissues perpendicular to the direction of the applied force. Pre-tensioning through circumferential strain was also thought to have played a role in maintaining the integrity and resistance to Brazier buckling of the neck tissues by resisting structural failure via ovalisaton. As onion development approached tops-down, the ovalisation of pseudostem bases under transverse load increased, demonstrating that resistance to deformation had decreased. Residual circumferential strain also declined from the beginning of canopy collapse providing further evidence of lowered resistance to structural failure, and therefore Brazier buckling. It was posited that the decline in residual strain could be due to a decline in turgor and/or tissue mechanical properties *per se*. In this study, cell turgor was inferred by measuring osmotic potential of highly turgid and expanding bulbs and pseudostem bases. Osmotic potential declined in both the pseudostem and the bulb tissue but did not differ between the two tissues. This indicates that the softening of

pseudostem tissue was not a result of changing water relations, but instead suggests a change in tissue mechanical properties. Mann (1952) reported that in garlic parenchyma cells on the inner side of the leaf sheaths that comprise the pseudostem degenerate first during senescence. If this is true of onions this mechanism would allow for the continued redistribution of assimilates through the intact vascular tissue during and after pseudostem collapse. Further histological investigations of the leaf sheaths within the pseudostem are recommended in order to test this hypothesis.

Softening of the base of the pseudostem was quantified using a custom designed testing platform (Chapter 4 and 5). To the knowledge of the author, this is the first study to quantify the softening of onion pseudostems. This objective procedure used a 4 N load to quantify resistance to pseudostem ovalisation and has several advantages over the subjective visual estimates of crop development used by industry. For instance the early, measurable change in softening prior to any lodging in the crop (Chapter 4 and 5) could be used for monitoring crop development and scheduling lifting in commercial production. This change could be used in conjunction with the current standard marker for growth development, tops-down. In addition, the softening of pseudostem tissue could be differentiate development within and among cultivars (Chapter 5). The testing platform was built at low cost (<$200AU) and was simple to use, though some training may be required for users, and assessments take longer in comparison with a general assessment of tops-down percentage within a crop. The prototype device was found to be effective at measuring pseudostem softening in both laboratory and field environments.

Does crop growth rate effect storage potential?

When agronomic treatments were used to modify onion growth rates in two commercial crops, bulbs which received additional nitrogen applications were found to be softer than controls after long term storage. Therese differences were not detectable at harvest and show that some of the effects of these agronomic treatments may only be apparent after long term storage. On the other hand, sprout growth was unaffected by the agronomic treatments. Furthermore, differences in bulb firmness at harvest were recorded between crops, however these differences diminished with long term storage. The differences between crops and the effect of treatments were independent of bulb size, and indicate that field factors other than those investigated contributed to bulb firmness and were not attributable to bulb growth rate *per se*.

The growth modifying treatments changed the allocation of resources between foliage and bulb. When additional nitrogen was applied or foliage removed during early crop development to affect growth rate, a higher proportion of dry matter was partitioned to foliage in comparison with the control treatment (Chapter 3). This is consistent with previous findings that additional nitrogen fertiliser can affect growth rate and increase partitioning of dry matter to leaf blades (Brewster 1990). Importantly, this resulted in plants with heavier canopies and smaller bulbs prior to lodging. Though some of this balance may have been corrected by remobilisation of assimilates from the foliage to the bulb (Chapter 3 ; Nilsson 1980), redistribution was insufficient to compensate for the smaller bulb size prior to lodging.

Consequently lower bulb yields were observed when the plants were lifted and bulbs cured when compared to the control.

The evidence from the growth modifying treatments and those from the time of lifting experiments show that crop agronomy can have a significant impact on the post-harvest storage potential of onion bulbs. The storage potential of these bulb can also be affected by postharvest handling.

Do impacts to bulbs during grading affect storage potential?

Onions that passed across a commercial topping and grading line were found to have reduced storage life, however, the magnitude of the response depended on the crop that bulbs were sourced from. Bulbs with a relatively low metabolic activity were less responsive to impacts from the topping and grading processes, demonstrating that differences existed among crops prior to topping and grading. This finding indicates that much of the storage potential is determined prior to topping and grading and that respiration rate is a potential proxy for estimating how bulbs will respond to impacts during handling. The concept of using respiration rate as an indicator of post-harvest storage potential has previously been considered for species with shorter storage life and higher respiration rates such as sweet cherry (Sekse, 1988.) and kiwi fruit (East et al. 2007). The range of respiration rates among crops and the relationships described between impacts and post-harvest storage indicators suggest that there may be value in exploring this concept further on onions.

Does the location and magnitude of physical impacts affect onion storage life?

Bulbs were found to be most sensitive to physical impacts to their base plate. The identification of greater sensitivity of onions to base plate impacts is unique to this study and indicates that impact location should be considered when designing harvesting and handling equipment, and when planning future studies to assess the sensitivity of bulbs to physical impacts. Bulbs impacted on the base plate exhibited higher post-impact respiration rates, sprout growth rates and, at the highest impact magnitudes, decay and death of the meristematic and inner scale tissues were observed.

Cell rupture was noted in tissues of the firsts two scales directly below impact sites to the equator, but not from impacts to the dense cellular composition of the base plate. In the absence of observable tissue damage, an explanation for the greater sensitivity to impacts to the base plate is elevated meristematic activity, and possibly undetected damage to these tissues. This elevation would not have been observable in studies which focused on the extent of tissue bruising (Timm et al. 1991) and is most important when bulbs are destined for long term storage.

Previous studies have examined drop height and load during storage (Herold et al. 1998; Timm et al. 1991) but did not control for impact location on the surface of the bulb. The findings of a positive relationship between impact magnitude and both respiration rate and

sprout growth in this study are in agreement with Herold et al. (1998), who also found increases in external sprouting. However, Herold et al. (1998) recorded only the presence or absence of external sprouts. The use of internal sprout growth measures in Chapter 3 may be preferable to the presence of external sprouting as it shows the progression towards externally visible sprouting and therefore the transition from dormancy to the second year of growth.

Respiration rate was used as a general indicator of metabolic activity within bulbs and has been used previously in onion (Benkeblia et al. 2000; Herold et al. 1998; Ward & Tucker 1976; Yasin & Bufler 2007) and other crops (Burton et al. 1987; Burton & Schilte-Pason 1985; Moretti et al. 1998; Saltveit 2000). Respiration rates initially peaked and then rapidly declined 1-2 days post impact. This was followed by a slower decline to a relatively stable resting respiration rate which then gradually increased over the following months. It is unclear what biological processes are driving this latter increase in respiration rate, however it can be speculated that this is part of the transition to sprout growth and development within the bulb (Yasin & Bufler 2007). Bulbs with higher respiration rates lost more weight and a greater number of bulbs were discarded due to internal breakdown or external sprouting in these high respiration crops. If under further examination the relationship between respiration rate and storage life is consistent, there may be the potential to screen crops based on respiration rate to predict storage potential without reliance on visual inspection alone. Thus, exporters could allocate crops with the greatest storage potential to markets with the greatest storage life requirements.

From a commercial export perspective, handling systems attempt to minimise the number and magnitude of physical impacts however, collisions are not completely mitigated and equipment does not control bulb orientation. Therefore most handling systems likely contribute to some extent to reduced bulb storage potential. Furthermore, uncontrolled orientation would lead to greater bulb to bulb variation in storage life within a crop as impact location can be assumed to be random (Chapter 2). Continuing to reduce physical impacts and implementing measures to control of bulb orientation during grading and packing processes has the potential to improve the consistency of bulb quality following long-term storage.

Conclusions and recommendations

Three findings from this work stand out as novel contributions to the body of scientific knowledge. First, the importance of the location of mechanical impacts on the respiration rate and internal sprout growth of onion bulbs in storage. Second, the identification of *ca* 90% tops-down as the optimal time to lift onion crops for maximising storage potential in the Tasmanian production system. Third, establishment of pseudostem softening as the regulatory mechanism of canopy collapse and development of a process to quantitatively measure the probability of canopy collapse. This knowledge may facilitate improvements in the scheduling of crop lifting within temperate climates to optimise the trade-off between yield and storage potential.

The core research objectives of this study were to derive new information to inform management of onion production and post-harvest handling for export onion crops. We recommend the Tasmanian onion industry should:

- Minimise the number and magnitude of physical impacts to bulbs during harvesting and handling;
- Avoid impacts to bulb base plate during mechanical handling;
- Consider the location of physical impact(s) in all future assessments of bulb impact sensitivity;

- Lift bulbs at ca. 90% tops down to achieve the best trade-off between long-term storage potential and yield;
- Take into account lower storage potential of crops or sections within crops that are not lifted at *ca* 90% tops-down during subsequent storage and handling;
- Monitor and evaluate the ability of field staff to predict tops-down percentage and the tops-down percentage at which lifting occurs;
- Use pseudostem softening to track crop progress towards tops-down to aid in the scheduling of lifting;
- Consider further improvements to the pseudostem softening testing platform including; modifications to allow the repeated *insitu* measurements and automation of elements of the testing apparatus;
- Target uniform crop maturity through agronomic management to minimise variability within-crop storage potential;
- Consider measuring bulb respiration rate (CO_2 evolution) in storage as a screening tool to identify container lots with potentially low storage potential;
- Further investigate internal sprout growth and its relationship with skin fracture to reduce market rejection;
- Consider using double haploid seed lines (despite low storage potential in comparison with other cultivars) in future studies to reduce genetic variability (Revanna et al. 2013);

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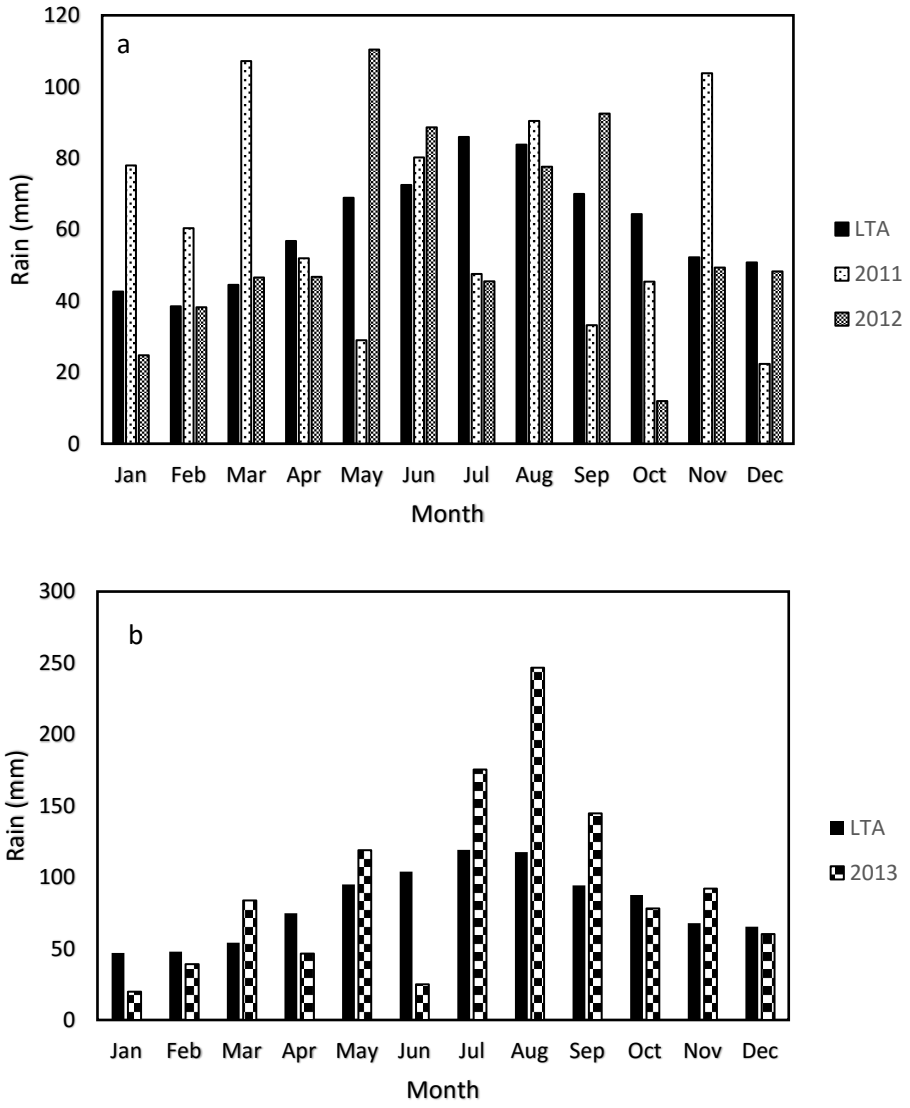
Appendix



Appendix 1. Mean monthly long term average (LTA) rainfall over the period 1900-2016 and monthly rainfall for years 2011 and 2012 (a) Hagley and (b) Forthside. Obtained from the silo weather data system. (http://www.longpaddock.qld.gov.au/silo)